

New machinery for UK science

After only a year, the new-style administration of British academic science has more enemies than friends. The best course would be to redesign the machinery, the best hope is that it will be improved.

THE British, humiliated earlier this week at the English national sport of cricket by a bunch of skilful Caribbeans, seem also to be heading for defeat in their next most gripping preoccupation, that of reorganizing the administration of publicly supported science. Although new arrangements for deciding how the budget for basic science should be distributed were introduced only at the beginning of 1983, the new system is already in trouble. The focus of general discontent is the Advisory Board for the Research Councils, the committee whose indispensable function is to tell the Department of Education and Science how the annual science budget for academic and basic research should be divided among its traditional recipients — five research councils, the Royal Society and the British Museum (Natural History).

For much of the time since the early 1970s, the board was a cosy committee of the executive heads of the research councils, the chief spenders of the science budget, accompanied by the chief scientists of other government departments. A select committee of the House of Lords then complained that the board had become too ingrown, too idle and too unreflective. The government declined to follow the advice then given that there should be a part-time minister with separate responsibility for civil science, but instead agreed that the board should be strengthened (which in practice has meant enlargement) and encouraged to take a wider view of the conduct of civil science. A year ago, those involved were full of hope that the new arrangement would function well. What has gone wrong?

Complaints abound, not the least of which is that the board has been turned into yet another talking shop of all the talents. Certainly it is too large to take decisions, or even to give advice decisively. The new chairman of the Economic and Social Research Council, Sir Douglas Hague, seems subversively to have been making this point to his colleagues in the past year, and cogently. To say the least, it is a sign of weakness that the board, for all its distinction, when faced with the question raised by the Science and Engineering Research Council whether the United Kingdom should continue to contribute towards the CERN high-energy physics laboratory, could not decide off its own bat but had instead to set up yet another committee, prolonging uncertainty by at least a year. Another difficulty that has arisen is that the research councils, whose chief executives remain members of the board, must turn themselves into supplicants of their lay (and less expertly informed) colleagues when they bid for their shares of the cake (most recently at the meeting on 7 June). It is galling to those concerned that the advice eventually given to the Secretary of State for Education and Science should be based on only second-hand knowledge of what the councils actually do with the money they are given to spend. The difficulty is especially acute when, as now, marginal changes in their budgets may determine the survival of particular laboratories.

Benefits

Before resentment of the present arrangements gets out of hand, its benefits should at least be acknowledged. For the past two years, the board has operated more publicly than previously, publishing both its advice on how the budget should be divided and (earlier this year) its sour response to the denial of extra funds. With luck (and time), it could yet become a means by which the British research community's sense of how limited resources

should be used could be channelled more effectively into the conduct of public policy on civil science. The board has also, with its tiny and poor secretarial resources, commissioned some useful studies on questions such as the relationship between research institutes and universities, and between university research and industrial sponsorship. In passing, as part of its cake-cutting, the board has also pushed the research councils towards a greater concern for university research than for their own institutions. And while there is no prospect that, on present form, it will become collectively so knowledgeable about the pattern of British civil science as to refute the research councils' charge that it is inexpert, the need for such an all-knowing institution is itself questionable.

These frustrations would no doubt have been borne more easily in other, happier, times. If the British science budget were still growing every year, the great and the good would be able to supplement their advice to the Department of Education and Science with a few urbane comments on the opportunities lying ahead. Bad tempers have made their appearance only because those who spend the science budget, chiefly the research councils, are unable to satisfy the demands now being made on them by researchers seeking support for projects that are eminently worthwhile. It does not help that this year, the board has been compelled to repeat the demand, refused last year, for extra funds, with only committee-style eloquence to help. But these are precisely the circumstances — likely to persist for many years — when an effective commentary on public policy for science is needed. The strains that have become apparent are indeed a proof that the system needs to be changed. Here are three directions for reform.

Reform

First, most modestly, the present arrangements could be made to function more effectively. To that end, the board should be given an adequate secretariat, one composed of people (not necessarily civil servants) who understand the problems it needs to tackle. While the essence of the board's task may be to cut the annual cake of public spending on science, it could then hope to do this more intelligently by finding out what happens to the funds that eventually find their way into the hands of the research councils. Many obvious questions are unanswered. How many universities have set up research committees, as the board asked them two years ago, to plot a strategy for their institutions' research? (Some say that the answer is just one.) Are public research institutes collaborating more effectively with universities now that they have been bidden to do just that a dozen times? (Some are, some are not.) What is the effect on the pattern of academic research in Britain of the stream of exhortation by the British Government in favour of this, and then that, brave new opportunity? (Mostly baleful.) And is it true that, for reasons that are as much psychological as financial, the British research enterprise has lost its capacity to compete with others? (Probably yes.) What are the prospects — and problems — of strengthening inter-European collaboration in ways that would economize in effort, not waste it? (Many, but unexplored.) Without means of dealing intelligently with these and related questions, the board continues to lose conviction — and friends.

Second, and more imaginatively, the board could be given wider terms of reference. In the curious British way, its sphere of interest is confined to scientific research supported by the



Department of Education and Science. Other parts of the public research enterprise are within the purview of a different committee (the Advisory Council for Applied Research and Development), which lately has not surfaced in public view. There is no committee to ask questions about the relationship between civil and military research. It would still need a better secretariat. It could deliver the annual goblet of advice to the Department of Education and Science more convincingly if it knew more directly what research is being sponsored by other departments. And it could avoid the present frustration by considering how the coordination between the disjoint halves of British public science could lead to greater effectiveness. In the real world, this is the most to hope for but the least for which to clamour.

Third, outrageously, is the remedy suggested in 1982 by the House of Lords: whatever apparatus there may be for giving specialized advice, let there be a minister specialized at receiving it. As things are, the British Government has built itself into a box. Lacking specialists within the civil service (as in West Germany) or an intimate relationship with the government's own specialist agencies (as in France), departments turn to advisory committees for advice which may be taken seriously when it is palatable and otherwise ignored. The downfall of the Advisory Board for the Research Councils can be traced to the occasion last year when it asked for more and was denied. Even if the Prime Minister rather than the Secretary of State was the one who finally said no, the doctrine of collective responsibility lumps the board in with all other dissident disappointed claimants on public funds — local authorities, schoolteachers and the unemployed. There is nobody to put the case, within the government, that the once and for all decline in the quality of the British research enterprise which is now on the cards will cast an even longer shadow. Within the political system to which the British are attached, but which allows ministers to carry only one torch at a time, only the designation of a single-minded political standard-bearer can enable the arguments for rescuing British science to be heard. □

Claims to antiquity

Publishers like to claim either antiquity or modernity. Cambridge can boast of both.

CAMBRIDGE University Press last week celebrated what it calls its 400th birthday with a "Garden Luncheon Party and Great Exhibition", offering its guests the presence of Prince Philip, the chancellor of the university, some sunshine, a band and a menu from which strawberries, usually mandatory on these occasions, were absent. Cambridge boasts that it is the oldest press in the world with an unbroken history. Only 80 years after Gutenberg, Henry VIII issued Letters Patent in 1534 granting the university "lawful and incontestable power to print there all manner of books". Although the first book did not appear until 1584 — even then, too long an interval to be explained as normal publication delay — it was a single but crucial year ahead of Oxford University Press and three years before the Vatican Press. Confusing for students of priority disputes was a 500-year celebration held by the University of Oxford in 1978. That occasion, Cambridge says, marked the quincentenary of the first book to be printed in the city of Oxford, not by Oxford University Press itself.

In science publishing, Cambridge University Press has been strong in mathematics and physics. In 1713, a revision of Newton's *Principia Mathematica* appeared under its imprint. Its other authors included James Clerk Maxwell, Rutherford, Jeans, Schrödinger, Einstein and, more recently, Hawking and Chandrasekhar. Undoubtedly the best known of the contemporary works is Joseph Needham's mammoth *Science and Civilisation in China*, Volume 6, Part 2 of which was published in April. Legally the press is unique, formally a department of the university with the status of a charity. It thus pays no tax. The press is governed by the 18 members of the syndicate, appointed by the university, which must approve all publishing proposals. Mercifully, this apparently anachronistic constitution seems not to be inhibiting. □

Unwelcome precision

Last week's US missile test could destroy the ABM treaty.

STAR wars are becoming uncomfortably realistic. That is one reading of the successful test last week of the US ground-based missile system, based on the Minuteman antiballistic missile, in which a dummy missile warhead was successfully intercepted within hours of a crucial decision by the US Senate about continuing support for these and related projects (see p.660). But last week's test was no part of the comprehensive defence against other people's ballistic missiles that President Reagan has been canvassing this past year. Instead, it is part of what some may call the conventional Anti-Ballistic Missile (ABM) programme which, in the United States and the Soviet Union, has its origins in the early 1960s. The fact that the test (the fourth) succeeded is nevertheless a sign that the treaties which are the fragile reminders of the time, not so long ago, when the superpowers felt able to sign agreements with each other may be coming to the ends of their useful lives.

In 1972, when the ABM treaty was signed between the Soviet Union and the United States, the openly acknowledged incentive was the fear that, without such a treaty, the deterrent value of strategic forces would be eroded. If one power believed that it could destroy the other's missiles, it would be less fearful of making a first strike with its own. That the two powers should thus openly acknowledge the identity of each other's calculations of the psychology of nuclear war was in itself a triumph. The treaty offered a little more — the promise of a respite from ruinously expensive development. But now those benefits appear to be receding. Both sides seem certain to be spurred by last week's test to further development. Technically, there is no reason why the new US system should not be stretched so as to become a second antisatellite (ASAT) weapon, capable of destroying satellites in low orbits, and thus a partner for the US Air Force system called MHV, launched from F-15 aircraft and already tested. (The traditional rivalry between the US Army and the Air Force will, left to itself, ensure rapid progress.) But technically, again, the arguments of the early 1970s remain valid that no rocket-borne system of this kind can be a certain defence against ballistic missiles with several warheads.

The first casualty will be the ABM treaty. The signatories accepted limitations on the testing of these weapons, although research was to be allowed. The signs now, however, are that unrestrained testing will create the impression, on one side or the other, that the treaty is a dead letter. Then, that is what it will become. And in such a setting, the prospects of an agreement on more elaborate systems to be deployed for longer periods above the atmosphere of the Earth will be even smaller than at present. The United States, after all, has not yet launched as many anti-satellite weapons as has the Soviet Union, which began to test its system as long ago as 1968. President Reagan will be able to certify to Congress whenever he likes that he has complied with the Senate's qualification of its grant of star-wars funds by saying that no agreement with the Soviet Union would be "consistent with the national security interests" until the United States has done at least as well. But by then, the Soviet Union will have provided further goals at which to aim.

The depressing reality of all this is that it will be at the least a waste of resources on both sides, and will be dangerous as well. While the star-wars idea is said (by some of those privy to what is planned) to be a technically interesting bag of innovations, nobody has been able to convince the sceptics that a star-wars defensive system could destroy a high proportion of the other side's missiles. And for as long as that remains the case, the course on which the Senate has allowed the US Administration to embark, and on which the Soviet Union is already engaged, cannot counter the objections raised against ABM defence in 1972: if there is a nuclear balance, it is bound to be upset. The irony, of course, is that the two governments were prepared to accept the logic in 1972. They should now follow suit for star wars. □

Australian uranium

Chance of policy change on nuclear fuel

Canberra

THE Australian Labor Government has been given an influential blessing for a change of policy on uranium exports. On 31 May, the Prime Minister, Mr R.J. (Bob) Hawke, presented to the federal parliament the report of a committee which argues that the mining and milling of uranium should continue and that the government should have authority to approve new mines and supply contracts.

If eventually adopted, the policy now recommended will represent a substantial change in the policy which the Labor Government has followed since its election. During the past year, the federal government has allowed existing operations and contracts to continue but has set its face against new enterprises. The new report argues not merely that the supply of uranium overseas should be increased but that Australia should participate in the enrichment of uranium, the reprocessing of nuclear fuel and, possibly, other steps in the nuclear fuel cycle.

These recommendations will strengthen Mr Hawke's hand in influencing uncommitted delegates to the Australian Labor Party (ALP) national conference next month, when strong opposition from the left is expected to the government's policy of allowing existing mining to continue, even under stringent supply conditions.

The 300-page report, *Australia's role in the nuclear fuel-cycle*, was prepared at Mr Hawke's request by the Australian Science and Technology Council (ASTEC), under the supervision of its chairman, Professor Ralph Slatyer, former Australian Ambassador to the United Nations Education, Scientific and Cultural Organization, when in November last year the government granted permission for the development of a new uranium mine at Roxby Downs, South Australia, potentially the world's largest.

The Slatyer report recommends that Australia, although not itself likely to use nuclear power, should accept that for many countries nuclear energy has become a fact of life. And since Australia owns 30 per cent of the uranium resources of the West, its contribution to nuclear non-proliferation should be enhanced by participation in the nuclear fuel cycle.

As a reliable long-term supplier of uranium, Australia would be in a position to influence the behaviour of other states, the report argues. Thus, by helping to ensure international energy security, Australia might reduce the motivation of other countries to engage in other steps of the fuel cycle, particularly reprocessing and fast-breeder development.

The compilers of the report are unconvinced that supplying uranium to nuclear weapons states frees other uranium for use in weapons (vertical proliferation). Denial of supply to such states, the report says, would be an empty gesture, scarcely affecting weapons programmes, since stockpiles are already adequate. Similarly, denial would not deter horizontal proliferation, since the political decision to develop nuclear weapons can in present circumstances be implemented by any country with sufficient determination and a reasonable scientific infrastructure. The absence of a civil nuclear industry would not prevent the manufacture of nuclear weapons. In any case, the report adds, such a country is unlikely on efficiency grounds to rely on civil power reactors for the manufacture of plutonium.

Other recommendations include continued support for a nuclear weapons-free zone in the South Pacific and for an Indian Ocean zone of peace. Australia should seek to join the board of governors of the International Atomic Energy Agency and spell out the feasibility of regional treaties with

South and East Asian countries along the lines of the Treaty of Tlatelco. The regime should be strengthened by internationalizing the steps in the fuel cycle, but sensitive facilities, particularly enrichment and reprocessing plants, should be located in as few countries in the region as possible, Australia and Japan for example. There should be support for the moratorium on ocean dumping of low-level waste, and continued research on the Australian-designed ceramic Synroc, the most promising high-level waste form, but Australia should not be obliged to dispose of high-level wastes from Australian uranium.

For the government and Mr Hawke, everything now hangs on the outcome of the Australian Labor Party's national conference, which seems unlikely to endorse a uranium policy going beyond that now defined by the government party caucus at Canberra and which is opposed to further uranium mines after Roxby. A further difficulty for the government stems from the ban on uranium sales to France, intended as a protest against French nuclear tests in the Pacific, the latest of which took place on 17 June. Unless the tests end, the Labor Party is unlikely to relax its traditional view that Australian uranium should not be used for other people's bombs. This, the government fears, may lead to trade reprisals and even to a law suit at the International Court of Justice.

Jeffrey Sellar

US-China nuclear treaty

Congress makes difficulties

THE United States-China nuclear exchange agreement which President Reagan announced triumphantly in Beijing last April is almost certain to be postponed and may fall through altogether. The text of the agreement initialled in Beijing has not yet been submitted for congressional approval, and the administration is now conceding privately that the assurances on non-proliferation may not be explicit enough to satisfy Congress.

A State Department spokesman said last week that the text had been studied by the relevant federal departments, including the Nuclear Regulatory Commission, and found to comply with US law on the transfer of nuclear technology. But he also revealed for the first time that the administration had sought renewed talks with the Chinese Government "to ensure a full mutual understanding".

What appears to have happened is that the administration was so eager to close the deal while President Reagan was still in Beijing that it fudged the two issues that had already dragged out the negotiations for more than two years. One is the status of China's promise not to help other nations to acquire nuclear weapons; the other is a requirement that China must seek US approval before using nuclear fuel from American-supplied reactors in nuclear

warheads or for warship propulsion.

Persistent allegations in Congress that China has assisted Pakistan in a nuclear weapons programme have been a major reason for Congress's lukewarm attitude. Last week, however, the *Washington Post* published a startling news story maintaining that the US delegation in Beijing had decided to accept verbal assurances on non-proliferation from Premier Zhao Ziyang instead of insisting on explicit written assurances in the text of the agreement. There had previously been reports that the thorny problem of China having to ask for US permission to use reprocessed fuel from US-supplied reactors had been overcome by "clever drafting".

Just why the administration has had second thoughts about the adequacy of the safeguards it claimed to have negotiated successfully last April is a mystery. Officially-inspired leaks suggest that new intelligence information has confirmed China's role in Pakistan's nuclear weapons programme.

It is just as likely that the Nuclear Regulatory Commission and other agencies with close experience of Congress's views on nuclear proliferation have concluded that the agreement is too vague to be approved in its present form.

Peter David

Electron accelerators

Southern US project delayed

Washington

THE group of US universities unexpectedly selected last year to build a major new electron accelerator has begun hesitantly. After a year, the project still has no director and, in the absence of a definite estimate of construction costs, Congress is balking at appropriating the full amount requested by the Department of Energy (DoE) for the project, now known as the Continuous Electron Beam Accelerator Facility.

The Southeastern Universities Research Association (SURA), a consortium led by the University of Virginia, was chosen last year by the Nuclear Science Advisory Committee (NSAC) to build the new machine at a site in Newport News, Virginia.

The decision set off a long political battle when Argonne National Laboratory, which had hoped to win the committee's recommendation, took its case directly to Congress and the Secretary of Energy. Traditionally, the physics research community has accepted as final the decisions of NSAC, which is made up of nuclear physicists and is formally an advisory body to DoE.

The fight between Argonne and SURA may have played a part in the slow start of the project. James McCarthy, a professor of physics at Virginia who is acting director of the accelerator, said that last year's fight gave the project a "political visibility" which provided greater congressional scrutiny, even after the Secretary of Energy had settled the matter last summer in SURA's favour.

The House of Representatives approved the administration request of \$5 million for research and development and \$2 million for construction in fiscal year 1985, but the Senate, complaining about not having a detailed cost estimate or a clear justification for the project, cut the figures to £2.5 million and zero respectively. At the bidding of Senator Bennett Johnston, the senior Democrat on the Senate Energy Committee, the Senate also asked DoE to provide a five-year plan for nuclear physics that would include the options of going ahead with the SURA accelerator or dropping it.

Although SURA would still be allowed to go ahead with the detailed architectural and engineering design work it had called "construction" in its budget request, the Senate's elimination of construction funds serves notice that the future of the project is by no means certain. It is generally difficult to kill a project once it has been accorded a line-item construction authorization.

SURA originally proposed a budget of \$20 million for next year, but backed off in the face of congressional complaints that the ultimate cost is uncertain. SURA's original proposal was that the total cost

would be about \$100 million, but NSAC, considering that unrealistic, increased it to \$150 million. In testimony to Congress this spring, McCarthy mentioned a figure of \$225 million. Although most of the increase can be explained by inflation, there appeared to be some new items (such as increased office space).

Some of the Senate's caution may stem also from worries about whether the United States can afford such projects if it is to go ahead with the massive proposed Superconducting Super-Collider, which may cost \$3,000 million.

D. Allan Bromley of Yale University, who chaired the NSAC panel that recommended SURA, said that the physics community still considers the project to be a first priority. He said that although there is widespread disappointment that more progress had not been made, much of the trouble is a result of a "Catch-22" dilemma; SURA cannot attract a director without assured construction funds, and cannot get assured construction funds until it has a director and a definite design.

According to McCarthy, an offer has been made to a "leading accelerator physicist", who will decide by 1 July whether to accept the directorship. That date is also when SURA will receive \$1 million appropriated by the Virginia legislature and an additional \$1 million that Congress has just allocated to DoE to "reprogram" the project. The funds will allow SURA to begin filling 9 senior and 15 junior positions on the scientific staff.

Stephen Budiansky

Academic ethics

Perils of scholarly plagiarism

Palo Alto

A distinguished Stanford University professor resigned as chairman of the department of medicine last week after a university ethics committee found him guilty of "grossly negligent scholarship". Dr Kenneth Melmon, who keeps his faculty position, was censured for plagiarizing roughly a quarter of a chapter in an endocrinology textbook. The committee said that no fraud had been committed and that Dr Melmon had had no conscious intent to deceive.

Trouble arose when the publisher of another medical text, *The Pharmacological Basis for Therapeutics*, found that large portions of that had appeared in Dr Melmon's chapter contributed to the endocrinology book (see *Nature* 5 April, p.489). At the time, Dr Melmon said his friend and editor of the endocrinology book, Dr Robert Williams, had urged him to use portions from the pharmacology book, promising that permission and at-

Computer theft boom

Washington

A SURVEY of US companies and government agencies has provided the first systematic evidence of how widespread computer crime has become. Of the 283 organizations that responded, 72 reported "known and verifiable" losses as a result of computer crime in the past 12 months. The average loss was in the range of \$2-10 million, but one organization reported losing more than \$100 million.

The survey, conducted by the American Bar Association, defined "computer crime" quite broadly to include embezzlement, theft of software and even the unauthorized use of computers for personal programming. The last was found to be the most common "crime", reported by 62 respondents; next in line was the theft of software, reported by 45, and use of computers for theft of assets (44).

While it is impossible to extrapolate the survey's results, the bar association states that "given the small number of organizations reporting these large losses . . . the total loss figure nationwide would appear to be enormous." The survey was sent to 1,000 organizations. Two-thirds of the identified perpetrators were employees of the organization, most often computer programmers.

The bar association called for the enactment of federal legislation against computer crime to aid in the reporting and prosecution of such incidents. Existing statutes that may be applicable, such as wire fraud, trespassing and embezzlement, carry penalties that the association said are often "far from commensurate" with the seriousness of computer crimes.

Stephen Budiansky

tributions would be secured. Dr Williams died, however, while the endocrinology book was being edited and his personal papers were later destroyed.

The Stanford ethics committee now says that it accepts Dr Melmon's explanation but has nevertheless censured him for his "recklessness" and "carelessness in dealing with the intellectual properties of others". He violated "the fundamental norms of the academic profession".

Dr Melmon was responsible for ensuring that proper attribution was incorporated into the chapter, the committee says; he should have checked that it was done properly.

Dr Melmon said he was resigning his chairmanship because "the mistake was a serious one". A clinical pharmacologist, he holds the Bloomfield endowed chair in Stanford's department of medicine and is a member of the Institute of Medicine of the National Academy of Sciences.

Sandra Blakeslee

Japan's unemployed PhDs

Unused researchers to arms

Tokyo

JAPAN's ever-increasing numbers of unemployed postdoctoral researchers are banding together to find ways of making life a little easier. But there is little they can do to increase their chances of actually finding an academic job.

The number of "overdoctors", or "ODs" as unemployed postdoctoral researchers are known in Japan, is now at an all-time high. (Overdoctor is an apt English word which the Japanese have invented for themselves.) There are at least 5,000 waiting for jobs in university departments, many of whom have now been waiting for more than ten years.

Japanese universities, unlike many of their Western counterparts, officially allow students who have completed their doctorates to stay on in their laboratories (upon payment of a monthly fee of around £40) to continue unpaid research while looking for a job. In some cases, such as the physics department of Kyoto University where there are now over a hundred, they outnumber the full-time teaching staff. Physicists, particularly theoretical

physicists, have been hardest hit, with more than a thousand ODs nationwide, followed by humanities, social science and agriculture.

To ease their plight, Dr Teruhisa Komatsu, an oceanographer from Kyoto University, has set up an OD Society and has found himself 200 members in just a few weeks. The society's chief aim is to keep ODs going, both financially and psychologically, while they are looking for *arubaito* (part-time work, this word stolen from the German *arbeit*) — usually teaching in a cramming school, translating technical reports from European languages or helping with the sophisticated analysis of university entrance requirements needed to help high-school students to pick a suitable university. The society plans to help find the best jobs for its members and also to remove the ODs' sense of isolation by arranging conferences for the presentation of their work.

Life as an OD is by no means easy. Although most ODs are in their late twenties, surveys show that only a little over half are married (compared with nine-

tenths of the general population of the same age). Average incomes are exceptionally low, only a little over £3,000 a year before tax.

A particularly cruel irony faces those who never find academic jobs. During their postgraduate study (two years for a master's course followed by three years of doctoral research), students must pay their own fees of around £400 a month as well as their own living expenses. There are no cosy European-style grants to cover the cost of education. As a result, almost all students take loans from semi-govern-



mental bodies. While the loans need not be repaid by those who find a university job, the unemployed are left with a huge bill for their education.

Neither the Ministry of Education, Culture and Science (MESC) nor university staff are taking steps to ease the OD problem, mainly because everyone lays the blame at someone else's door. One ministry official sums it up by saying that "they (the ODs) should seek work in practical, non-academic fields, instead of keeping their sights on a life in the ivory towers".

Certainly it is a common view that ODs bring about their own problems by "refusing" to work in industry, insisting instead on waiting for the impossible. There is some truth in this charge. Among physicists, for example, there are many who have had experience of computer programming and are in demand by industry. But it is nonsense to suggest that most ODs can easily find work of any other kind than the part-time work they already do.

Industry in Japan has a strong preference for graduates fresh from university, who can be trained in a company's own ethos. Even school teaching is hard to enter, with an upper age-limit for new entrants of around 28–32. Particularly hard-hit are the many ODs with training in agriculture, for there is no related practical field into which they can go. Small wonder, then, that the two most favoured routes out of the OD trap are a fellowship to a US or European university — and journalism.

The only long-term Japanese solution must be an expansion of the universities, but this is a political question. And the government intends to squeeze all the ministries again next year (with the exception of defence). Hopes of university expansion must for the time being remain in the realms of fantasy. **Alun Anderson**

Academic demography awry

THE OD problem results from the massive expansion of the universities in the late 1960s, which created many new doctoral schools. With the oil shock, the expansion of the universities has been halted, but there are fewer job opportunities for researchers from the new schools.

So why cannot the Ministry of Education, Science and Culture (MESC) reduce the flow of graduate students, or at least redirect them into areas, such as computer programming, where highly-trained people are needed? Unfortunately, the Japanese system is far from flexible. Once permission has been gained for the establishment of a doctoral school, there is little further MESC can do to control it. As no grants are given directly to students (and loans are easy to obtain), it is hard to redirect students quickly into new subject areas. And professors, enjoying considerable power under the "chair" system, will fight tooth and nail to prevent government meddling in their affairs.

Thus arises a common view within MESC that professors worried about the OD problem should voluntarily reduce their student intake and coordinate activities with others in their field to take account of future forecasts. Some professors have indeed done exactly this. But because teaching loads are high, it is virtually impossible for any ambitious professor to carry out a research programme without graduate students to help.

The contraction in the universities that has provoked the OD problem has also

caused another, potentially more damaging, academic problem, one which MESC seems again to be ignoring. The slowdown in the recruitment of new researchers has seriously distorted the age structure in the university research community. Simulations conducted by Kenichi and Kaoru Aoki of the Kyoto University Research Institute for Fundamental Physics show that, by 1991, the median age of university staff will peak very sharply at 48. By 2001, the distribution will be bimodal with the larger peak at age 58.

European countries, particularly West Germany, are no strangers to this problem. The difference is that in Europe studies are being made by official bodies rather than being left to private individuals and steps are being taken — such as the United Kingdom's "new blood" scheme — to ensure that there are enough young workers at universities.

Drs Aoki hold that Japan just does not take a long-term view of scientific research planning — day-to-day economics are the prime determinant.

Postdoctoral fellowships, at present non-existent in Japan, could bridge the immediate shortage of young university workers until retirements begin to ease the situation. The Aokis claim that with such fellowships, an average expansion of 1,800 university posts a year, well below the 1960s peaks when 5,000 new jobs a year were being created, would give the universities a very healthy age structure. **Alun Anderson**

Weapons in space

ABM test persuades US Senate

Washington

LAST week's interception of one Minuteman missile by another at an altitude of more than 100 miles was a feat of exquisite political timing. The successful test was announced by the Department of Defense at the beginning of a week in which the Reagan Administration's plans for testing a new antisatellite (ASAT) weapon and stepping up research on the proposed "star wars" nuclear defence system faced major congressional hurdles. In the event, the Republican-controlled Senate agreed to give the ASAT test an amber light. And Congress now seems certain to give the administration much if not all of the \$2,000 million it wants in 1985 for research on ballistic missile defence.

Of the two space weapon initiatives, the more pressing from the administration's point of view is the ASAT test. The Air Force has already developed a sophisticated aircraft-borne satellite interceptor (see *Nature* 308, 576; 1984), but the House of Representatives voted last month to block tests against targets in space for a year or for as long as the Soviet Union continued a moratorium on tests of its own weapons. In both houses, there is strong feeling that talks on an ASAT ban should be resumed, despite the administration's insistence that no such ban could be adequately verified. And in a piece of deft timing of his own, Soviet President Konstantin Chernenko again urged the United States to negotiate an ASAT ban before it was "too late".

To discourage the Senate from following the example of the House, President Reagan's Senate allies arranged a closed session in which senators received intelligence briefings on the state of Soviet ASAT technology. They are said to have been told that, in addition to its well-publicized ASAT interceptor, the Soviet Union now has the means to cripple US satellites with a laser beam from an Earth station or by jamming their radio communications.

A day later, the Senate voted by 61 votes to 28 to allow the ASAT tests to proceed provided only that the President shows that he is trying to negotiate "the strictest possible limitations on antisatellite weapons consistent with national security interests of the United States".

The effect of the Senate resolution, which will have to be reconciled with the position of the House in a conference committee, is to require some action by the administration that demonstrates a wish to prevent an arms race in space. But it is clear from the mood of the debate that a symbolic gesture will suffice. As long as Mr Reagan certifies that the ASAT tests are vital to national security, and will not represent an "irreversible" block to negotiation, he will be allowed to test the new weapon.

Whether the President will take up the option in a hurry is another matter. The administration is becoming uncomfortably aware of growing public concern about the prospect of an arms race in space. So is the Democratic Party; its national chairman, Mr Charles Manatt, said last week that the presidential election in November will give voters a chance to ponder the "frightening choices" entailed in the administration's plans for the militarization of space. President Reagan is under pressure from his own party to show some flexibility on arms control before the election; by the end of last week the administration was already hinting that it might be willing to discuss some limitations on ASAT development.

The short-term ASAT question has not entirely distracted attention from "star wars". While Congress appears still to be willing to underwrite the preliminary research called for in the 1985 budget, organized opposition to the concept is gathering pace. A "national campaign to save the antiballistic missile (ABM) treaty" was launched this week by a committee of senior politicians and scientists, including former President Jimmy Carter and former defence secretary, Robert McNamara. In an opening statement, the committee claimed that the expanded star wars testing programme requested by the administration will "soon collide" with the restrictions imposed by the ABM Treaty.

Meanwhile, in a bizarre coda to the political debate, the technical debate over the feasibility of the star wars programme is becoming increasingly unfriendly. Deputy Defense Secretary Howard Taft has taken the extraordinary step of asking the Office of Technology Assessment to withdraw a technical memorandum on the subject written by Dr Ashton Carter of the Massachusetts Institute of Technology (see *Nature* 309, 485; 1984). Mr Taft claims that the memorandum, which said the prospect of a near-perfect defence against nuclear attack was "remote", had subsequently been criticized on technical grounds by four independent review groups.

The Pentagon clearly expects last week's Minuteman interception to cut some ground from under the feet of those who doubt whether a defence against ballistic missiles is feasible. However, most critics of the star wars concept have already acknowledged that the interception of nuclear warheads in midcourse is feasible. Their objection is that a nuclear attack would involve so many missiles and decoys that such defences would be overwhelmed. The Office of Technology Assessment study therefore focused on the prospects for destroying missiles in their boost phase, before they could spawn their multiple warheads. It concluded that most of the novel technologies required could be countered with relative ease.

Peter David

US Naval Observatory

Research at risk

Washington

THE US Naval Observatory, one of the oldest government scientific institutions in the United States, is in trouble, according to the National Academy of Sciences. Short of funds and overburdened by its role as a support agency, the observatory's basic research capability is in jeopardy, according to a new study. Some divisions of the observatory are "one man deep", according to Lee Hunt of the National Academy staff, and are "struggling to provide the bare minimum of services". Scientists often have to act as administrators and technicians as well, all of which leaves little time for basic research.

The primary mission of the observatory is what it always has been: providing data to aid navigation. Much of its work at present, for example, involves calibrating



atomic time standards and carrying out the observations needed to adjust the International Atomic Time scale for astronomical and navigational use.

But the observatory has also established a solid scientific reputation. The study notes the observatory's longstanding work on visual double stars, now in jeopardy for lack of manpower, and its determinations of parallaxes of faint stars — data that "effectively define our knowledge about luminosities, colours and tangential velocities of faint stars in the neighbourhood of the Sun". "Too much of the astronomers' time must go toward technical support activities", the study says, but Hunt put the point more directly: "You find directors sweeping floors."

The shortage of manpower is apparent in the study's recommendation on the double-star programme. If "one other person were able to take over clerical jobs", the study says, "the staff would be able to do much needed work on new technology." Hunt said that the study panel felt that continued excellence in basic research was vital to attract qualified scientists and to keep them in the mainstream of science.

The observatory has suffered not so much from budget cuts as from benign neglect. But the consequences are just as serious, the academy panel said. "No action at this time is a decision to erode the quality of staff and the services of data and information it provides."

Stephen Budiansky

UK medical research

More MRC units threatened

THE parlous financial position of Britain's Medical Research Council (MRC) is obliging the council to reconsider the future of any research to which it is not "irrevocably committed". The futures of three research units supported by the council are now described as "under review" following critical reports by visiting parties that, in some cases at least, recommended the council to consider closure.

The council said that a decision had been taken "in principle" to close its unit of neurochemical pharmacology at Cambridge (see *Nature* 7 June, p.486). But, as if to advertise that British medical research is not completely moribund, the council has also announced a new research unit to undertake laboratory studies of tuberculosis.

Surprisingly, the location of the new unit has not yet been finally decided, although the probable choice will be the Royal Postgraduate Medical School at Hammersmith, London. The new unit will replace two existing units in London, one at Hammersmith and one at the Brompton Hospital, Fulham, which are to be closed when their directors retire shortly.

The formation of the new unit signals a shift away from the study of the effects of tuberculosis on communities and of chemotherapy towards the use of new techniques, including recombinant DNA technology and monoclonal antibodies. The director is to be Dr Juraj Ivanyi, now head of experimental immunobiology at

Wellcome Research Laboratories.

The three units whose future is uncertain are the Biostatistics Unit at Cambridge, the Pneumoconiosis Unit at Llandough Hospital in Penarth, South Wales, and the Mineral Metabolism Unit based in Leeds. A report by a regular visiting group to the Pneumoconiosis Unit has recommended closure, and the matter is to be considered shortly by MRC's Physiological Systems and Disorders Board. There has been a cloud over the future of the unit for some time, but the recommendation to close it was still a surprise to staff, who say they will be able to enter pleas for continuation when the board's recommendations, together with the unit's responses, are formally considered by MRC. The unit has no director in post at present, thus facilitating any closure decision.

The Mineral Metabolism Unit is also without a director, but its acting director, Dr M. Peacock, seems unwilling to accept that closure is one option to be considered by the disorders board. The unit's work on osteoporosis and adrenal stone disease is in line with recommendations of an international review of calcium in metabolism, and Dr Peacock is confident that MRC will find "no scientific grounds" to close the unit, although there have been some recent staff reductions. Much the same applies to the Biostatistics Unit, where interviews for a replacement director for Dr I. Sutherland have been called off at short notice.

Tim Beardsley

No sale yet for UK chip-maker Inmos

THE British Government is apparently still undecided about how it should introduce private capital into Inmos, the state-backed semiconductor manufacturer. The company, which started to trade profitably in the final quarter of 1983, has been provided with government capital of £65 million and loans and guarantees worth £40 million, and the Secretary of State for Trade and Industry, Mr Norman Tebbit, is keen to reduce the strain on the public sector borrowing requirement.

Earlier this month, Mr Kenneth Baker, Minister for Information Technology, said in the House of Commons that the government had vetoed a private share placing organized by the company that would have raised £30 million, on the grounds that the terms were not sufficiently attractive. Mr Baker's answers to supplementary questions made it clear that the government is anxious that Inmos technology should continue to be accessible to British industry and that there should be a commitment to continued development and expansion of Inmos's activities in Britain.

More recently, Thorn EMI has made

public its offer of £10 million for a share of the company, an offer which Inmos's chairman Sir Malcolm Wilcox described as "very interesting". But the matter is still under consideration by the Department of Trade and Industry, which oversees the nominal holder of public shares in Inmos, the British Technology Group.

The US communications group AT&T (American Telephone and Telegraph) is also now bidding again for Inmos. An approach by AT&T was rejected by the board of Inmos in February, but negotiations are now under way directly with the government. AT&T has, as a nod towards the principle of keeping some of Inmos's activity based in Britain, agreed that some of its research and development activity might be carried out by ICL, the British computer company. AT&T is understood to have made an offer of £45 million for the whole company, a figure regarded as derisory by Inmos management.

Inmos is now making a monthly profit of around £1 million and has great hopes for the future with its revolutionary "transputer" chip.

Tim Beardsley

Neural tube defects

British vitamin trial starts slowly

THE controversial British clinical trials of vitamin supplements in the prevention of spina bifida and other neural tube defects has begun, but on a much smaller scale than originally intended. A progress report issued last week said that so far the Medical Research Council (MRC) has recruited 132 women to the trial, still far short of the 2,000 target.

Even so, MRC says it is reasonably confident that recruitment will accelerate as new centres join the study. So far, physicians at a total of 16 medical centres in the United Kingdom and 12 overseas have agreed to take part, all with the approval of their local ethical committees.

The decision to conduct control trials with vitamin supplements was taken in December 1982, partly because of research by Professor R.W. Smithells at the University of Leeds which seemed to show that neural tube defects might be prevented by vitamin and particularly folic acid supplements before and immediately after conception. To separate the effects of folic acid from those of other vitamins, MRC advocated clinically controlled trials with randomly selected patients.

The trial has been controversial because the four groups of women to which volunteers are assigned at random includes one to whom only a placebo will be administered. During the past two years there have been complaints that the use of a placebo group is unethical in the face of *prima facie* evidence that vitamin supplements will prevent neural tube defects, and that it may even be a breach of the revised Helsinki code of 1975 on the use of human subjects in research. There is also some concern that the daily dose of folic acid being administered to one of the four groups in the MRC study is, at 4 milligrams per day, ten times that used in earlier work.

Recruits to the study, who must first declare themselves to their personal physicians, include only women who have already given birth to a child with spina bifida or encephaly and among whom the chance of a recurring defect is, at 3 per cent, comparatively high. MRC insists that the anxieties of protesters such as the National Childbirth Trust at the ethical basis of the trial are answered by its information leaflet for participants, which says that the consent of the woman's husband will normally be required and that women's medical care will not be diminished if they do not volunteer.

So far, 18 of the 132 recruits to the study have become pregnant (and one has produced a normal child). Whether the target number of 2,000 recruits will be found within five years is at this stage by no means certain.

Judith Abel

Artificial reproduction

Unrestrictive guidelines for UK

THE Warnock Committee's recommendations to the UK Department of Health on artificial reproduction began accurately to leak out last week. The main conclusions of the 18-month study, due to be published at the end of June, are that commercial surrogate motherhood agencies should be banned; that hospitals and clinics specializing in *in vitro* fertilization (IVF) and artificial insemination by donor (AID) should be licensed and required to register successful births; that the children born through AID programmes should be legitimized; and that research on embryos should be limited to 14 days after fertilization.

To administer these provisions, the committee recommends a permanent national licensing body whose role would also include monitoring embryonic research and advising government on new developments in artificial reproduction techniques.

On the issue of AID births (already accounting for at least 2,000 children in Britain each year), it has also been recommended that no sperm donor should be allowed to father more than ten children and that his identity should remain undisclosed; it is not yet clear, however, what subsequent access a child conceived by the AID method would have to information about his or her natural father.

The broad conclusions are not, however, unanimous. Two of the 16 members of the committee hold that surrogate motherhood should not be completely banned, but accessible only when there is no other option for a childless couple. The dissenters advocate that, in these circumstances, arrangements should be undertaken by non-profit-making bodies such as adoption and fostering agencies; the British Agency for Adoption and Fostering is not, however, enthusiastic about the suggestion.

On embryo research, two further minority reports (signed respectively by 3 and 4 members of the committee) are expected; one opposes on principle all research on human embryos, the other argues against the special creation of embryos for research purposes.

First indications are that the committee's report will be fairly well received by physicians and researchers, although some claim that the 14-day limit on work with embryos is too restrictive. (The Royal College of Obstetricians and Gynaecologists has recommended a 17-day limit.) The committee also eschewed a time limit on how long embryos can be kept frozen where parents desire several children, although the general specification is that it should be long enough to allow for children to be well spaced. Parents should be consulted on the fate of spare embryos, the committee says.

The leaking of the Warnock Committee's recommendations has been greeted with relief both by researchers fearing restrictive regulations and by those who see a need for swift legislation, especially with increasing commercial

British universities

Another turn of the screw

WHILE British universities contemplate the news that their recurrent grant is likely to fall in value by more than 2 per cent over each of the next 2 years, six of them have been named as guinea pigs for a study of university efficiency being undertaken by the Committee of Vice-Chancellors and Principals (CVCP) and the University Grants Committee. The chosen six are the Universities of Edinburgh, Essex, London (University College), Loughborough, Nottingham and Sheffield. These institutions will during the coming autumn collaborate in efficiency studies with a steering committee under Sir Alex Jarratt.

Although universities have been anxious to appear willing to cooperate in such efficiency exercises, their temper has not been improved by recent estimates circulated by CVCP which indicate that the recurrent grant is likely to fall in value by 4.4 per cent between the present financial year and 1986-87. The estimates are based on "indications" provided by the University Grants Committee and were arrived at by excluding the protected Information Technology and New Blood schemes and then incorporating government expectations for inflation over the next 2 years.

CVCP's document, circulated to vice-chancellors at the beginning of this month, argues pointedly that provisional levels of recurrent grant incorporate two levels of reduction in value: scheduled cash increases are lower than projected cost increases, and the cost increases are themselves lower than government expectations for inflation. The cash value of the recurrent grant is expected to rise from its current level of £1,249 million to only £1,320 million in 1986-87.

Mr Michael Baatz, secretary of the efficiency study group, emphasizes that the initiative for efficiency studies came from CVCP itself, albeit "in response to government interest". All British universities had declared themselves willing to take part, but the chosen six represent a variety of university types, old and new, large and small. The study is supported by a grant of £300,000 from the Department of Education and Science.

The plan for the study now agreed is unusual in at least two respects. Eight topics have been drawn up as suitable for investigation, ranging from the all-embracing ("financial management") to the

interest in surrogate motherhood.

Despite the sense of urgency in the scientific community and, indeed, among the Warnock Committee members, government legislation is not likely before October 1985. But there is likely to be mounting pressure on politicians to achieve some form of legal guidelines well before then, especially once the report is published, later this summer. **Judith Abel**

particular ("animal houses"). But only one topic will be investigated at each of the six universities included in the study (the topic to be chosen jointly by the university and the committee), and so at least two of the possible topics will be left untouched. And the committee is pledged to make no judgement on academic matters, such as what subjects should be taught.

Mr Baatz admits that there will be much discussion on what constitutes an "academic" matter. While questions such as level of provision for each subject are clearly out of bounds, the committee will hope to make recommendations on how resources might best be deployed to achieve stated aims. The academic side of these questions is being tackled by a separate CVCP working group on academic standards chaired by Professor Philip Reynolds, vice-chancellor of the University of Lancaster. **Tim Beardsley**

Reagan's Lysenkoism

US PRESIDENT Ronald Reagan should be awarded a special "Lysenko prize" for his "star wars" theories, Academician Evgenii Velikhov asserted last week. Velikhov, a vice-president of the Soviet Academy of Sciences and chairman of the Committee of Soviet Scientists for Peace and Against Nuclear Wars, was speaking on Moscow Radio's "English for North America" service.

According to Velikhov, Reagan's allusion to "new defensive systems" that would give the United States an antimissile capability was simply "Lysenkoism". Present-day lasers, he said, would have to be improved by ten million times to provide a reliable antimissile defence system. Like Lysenko, he says, Reagan is prepared to "put science into storage" if it gets in the way of his theories.

The hope of "protecting all people" is a "wonderful concept", but, like Lysenko's agricultural theories, is in reality "a lie" which cannot be implemented.

Velikhov says he made this clear during a visit to the United States last month, in meetings with the Federation of American Scientists, the US National Academy of Sciences and "people with political and military experience". **Vera Rich**

Baikal-Amur railway

Building too fast for efficiency

SOVIET plans to complete construction of the Baikal-Amur Mainline (BAM) railway by autumn 1984, a year ahead of schedule, have been jeopardized by a lack of understanding of the geology of permafrost. This year, the Soviet media have noted several instances where the rapid exploitation of geological research findings for the good of the economy, urged by successive five-year plans, has run into difficulties. Oil extraction from newly discovered fields in Siberia is far behind schedule, and it was revealed last month that the reservoir of the Cheboksary hydro-electric station on the Volga had caused an unexpected rise in the water-table between Cheboksary and Gor'kii, so that the water-table in Gor'kii has already risen to ground-floor level in some places.

Such problems seem endemic in a centrally planned system. Civil engineering works may be started without allowing sufficient time for preliminary surveys; and if one sector carries out a programme of "shock-work", others working at normal speed will appear to be lagging.

The trouble on BAM appears to be a result of the decision to bring forward the construction date, so that the last "golden link" could be laid to celebrate Revolution Day (7 November) this year. BAM, which has been hailed as one of the most important achievements of Soviet civil engineering, has been built in sections, and the running of trains on completed portions has already earned, its publicists claim, impressive sums from the Soviet economy. Looping far to the north of the (Tsarist-built) Trans-Siberian Railway, and thereby avoiding sensitive areas close to the Chinese border, BAM, when completed, is expected to offer fast and competitive freight rates for the container transport of goods from the Far East to Western Europe. The driving of the final link is therefore economically important.

The course of BAM was carefully planned — according to official reports — by a think-tank covering all the relevant scientific disciplines. The chief snag on the final choice of route was the Kodar range of mountains, through which a 2-km tunnel had to be drilled at an altitude of 3,000 m. When the completion date was brought forward, it became necessary to complete this tunnel by May 1984 (leaving six months for the track-layers to do their work), and the tunnellers forged ahead with such gusto that by 15 February, they had already excavated 1,600 m of tunnel.

Unfortunately, however, the concrete workers responsible for facing the newly dug surfaces made slower progress, and before they could catch up, lumps of rock began breaking away from the unfaced ceiling of the tunnel. It was eventually decided to reinforce the roof with metal structures, but this remedy was adopted

too late to prevent a serious cave-in.

Serious recriminations have broken out as to who was responsible for the cave-in. The tunnellers blame everything on the "mysteries" of the permafrost. They have hinted, moreover, that the strata encountered were less hard than the geologists had supposed, and that although temperatures were monitored in the excavated tunnel, readings were taken only at floor level, and it was only later discovered that temperatures near the roof were considerably higher (around 12°C). The chief directorate for the BAM construction, however, tends to blame "sluggishness" in

the concreting of the roof, while admitting that an elementary law of physics had been ignored — that at the temperatures that built up in the tunnel, permafrost gradually ceases to be permanent.

All the resources of Soviet society have now been mobilized: representatives of the Party bureau have been posted to "key sectors" and the concreting teams reinforced with Party cadres. The Ministry of Transport Construction has instructed the tunnellers to have the tunnel ready for traffic this autumn. The Chief Tunnel Construction Directorate, according to the Soviet newspaper *Sovetskaya Rossiya* considers this task "unrealistic". Officially, however, the schedule for driving the golden link remains unchanged.

Vera Rich

Optical microscopes**Reflector makes high price**

A RARE reflecting microscope constructed in 1827 by John Cuthbert under the guidance of Dr Goring, the eminent Scottish microscopist, was sold last week in Sotheby's London sale-room for £8,000, about three times the price expected. The instrument was based on one produced several years earlier by the famous Italian experimenter Giovanni Amici, but instead of varying the magnification by a set of eyepieces, Cuthbert has provided a series of elliptical mirrors and Lieberkuhns. The mirrors are mounted in tubes to which the Lieberkuhns can be attached.

The reflecting microscope first made its appearance when the struggle for the achromatization of the microscope had almost been abandoned. Great strides had been made in the mechanical properties but there had been scarcely any improvement in the optical performance of the microscope since its invention two hundred years earlier.

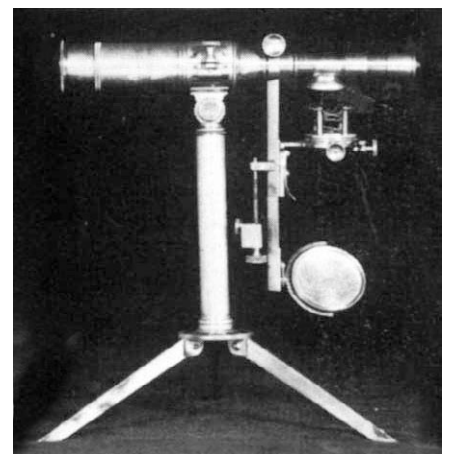
Isaac Newton had certainly not helped matters by supporting the view that the construction of a colour-correcting achromatic lens system was not possible, and in 1672 he wrote to the secretary of the Royal Society outlining his ideas on how chromatic aberration could be overcome in a microscope by the use of a "reflecting piece of metal instead of an object glass".

Newton's preoccupation with a reflecting rather than with a lens system was influenced by his conclusion that refraction is always accompanied by dispersion, an unfortunate conclusion which went unquestioned for many years and which resulted in considerable delay before serious research was undertaken on the achromatic lens. As to Newton's microscope, if he ever had plans for its construction, they appear not to have materialized.

The first satisfactory reflecting microscopes made their appearance some 50 years later, and were by the Italian and London makers, Amici and Cuthbert.

John Quekett, in his microscope treatise of 1848, points out the difficulties of making and using these instruments, but of the Cuthbert microscope, he says that it was (in its time) "the most perfect instrument manufactured in this country".

The success of Cuthbert's microscope was, however, short-lived. In 1830, just three years after its appearance, a development took place which struck a death-blow



The Cuthbert reflecting microscope

to all reflecting microscopes: the successful production of the long-awaited achromatic objective. For the first time, the conventional compound microscope began to emerge as an indispensable tool in science and industry. No longer was it merely a plaything for dilettante enthusiasts, who, to quote a nineteenth century historian, "regarded their costly instruments as precious toys rather than as tools for work and study".

Paradoxically the twentieth century has seen the re-emergence of reflecting systems for specialized use in ultraviolet or infrared regions of the spectrum; so even if Newton had been misled in his dispersion experiments, his pioneering work on the reflecting microscope was not altogether in vain.

Arthur Frank

Coronaries and diet

SIR — The article "You are not what you eat" (*Nature* 307, 199; 1984) gives the seriously misleading impression that dietary modification cannot reduce the risk of coronary heart disease. In addition, the article contains many inaccuracies.

In the Coronary Primary Prevention Trial referred to in the article, all patients received a standardized diet which limited saturated fats and cholesterol and which was designed to lower blood cholesterol by 3–5 per cent. Both the treatment and the control groups achieved a 4 per cent plasma cholesterol reduction as a result. It is wrong to imply that the control group (no drug treatment) could not have done any better; controls showing a large dietary response were deliberately excluded. More restrictive diets have frequently been shown to reduce plasma cholesterol levels by 10–15 per cent.

You are incorrect in implying that only those in the study who reduced their plasma cholesterol to a level near the national average benefitted. Reduction of risk was directly and continuously related to the per cent reduction in cholesterol concentration. (You state that the group receiving the drug (treatment group) reduced plasma cholesterol by 17 per cent; the correct figure is 13.4 per cent. And you state that non-fatal myocardial infarctions were reduced by 17 per cent; the correct figure is 19 per cent.)

Although you did not distinguish between dietary cholesterol and dietary saturated fat, your article carries the clear implication that diet is unimportant in reducing the risk of coronary artery disease. The results of the trial clearly showed the benefit of lowering plasma cholesterol. In fact, it is almost standard practice for the patient to be treated with diet first, and a drug added only if diet fails to achieve a satisfactory reduction in plasma cholesterol. The results of the Oslo study, in which dietary modification and elimination of cigarette smoking achieved reduction in plasma cholesterol and a significant reduction in coronary artery disease, are consistent with the results of the Coronary Primary Prevention Trial and with our recommendation that patients with plasma cholesterol levels greater than 240 mg dl⁻¹ should be carefully evaluated and should change their diet to reduce the level. Those whose plasma cholesterol remains elevated after a trial with diet are candidates for treatment with a drug. The suitability of this treatment must be an individual decision made by doctor and patient, based on accurate data on the patient's plasma lipoprotein levels. The trial indicates that vigorous treatment of this high-risk group will be effective if begun sufficiently early.

ANTONIO M. GOTTO

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Unfair on Illmensee

SIR — Your coverage of what you call "the Illmensee affair" continues to prove unfair. Or has it become common usage in *Nature* to report on the failure of anonymous "other researchers" who unsuccessfully try to "repeat Illmensee's experiments", as you do in *Nature* 308, p.394? Knowing Illmensee for more than sixteen years and having worked as one of his fellow PhD students in the same room for more than a year, I am fully convinced of his personal and scientific integrity. If others fail to repeat his work successfully — what of it? Even Carl Lewis has not yet reached Bob Beamon's 8.90 metres.

ECKHARD LIEB

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Statisticians cleared

SIR — Following the recent article on the fallibility of the polygraph (*Nature* 29 March, p. 449) and the correspondence it generated (*Nature* 17 May, p.203), it is clearly now time to update that apophthegm which is the bane of a statisticians' existence: "There are lies, damned lies and lie detectors"!

D.O. CROWTHER

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Indian items

SIR — Your news report (*Nature* 307, 307; 1984) rightly terms recent exhortations of the head of India's University Grants Commission (UGC) as utopian, although the UGC chief is to be credited for highlighting maladies responsible for academic backwardness in Indian universities. The bureaucracy and the politicians have strangled university autonomy. There is interference in day-to-day running of the academic administration, as witnessed only a few months back when the vice-chancellor of a university resigned in protest at a state political authority encroaching upon his stand to maintain discipline in the campus.

In India education is a state matter. Except with regard to research, technical and scientific education, the education is entirely within the purview of the states. For this reason education has not developed a national perspective, with each state going its own way. The first step to improve matters would be to bring education under the category of the concurrent list of the Indian Constitution. This will enable UGC to intervene effectively to protect academic interests.

In the Indian university system, a vice-chancellor has a very important role to play in shaping the academic life of a university. But the recent trend is to appoint vice-chancellors mainly on political grounds. So vice-chancellors have failed to inspire the

respect that students and teachers should normally have for a person who is supposed to be the leader of academic life of a university. In this context I may mention the report of Justice Mootham in which the choice of vice-chancellors was dealt with at length. Similarly, the Radhakrishnan commission on university education has not only described the quality of a person to be chosen as a vice-chancellor but also prescribed a procedure for appointment of vice-chancellors. Let academic merit be the guiding principle in the selection of a vice-chancellor. Furthermore, the highest authority in the government should be directly accessible to the vice-chancellor with no bureaucrat acting as a middleman.

Much of the erosion of university autonomy is done in the name of finances. This has reduced universities to just another section of the Education Department of the State Secretariat. There is no justification in placing a provincial civil servant designated as Financial Officer controlling the university finances. Such an officer has only a narrow bureaucratic outlook and cannot apprehend the need and priority of the academic challenges. Let the university be allocated "x" finances and details of its disbursement or priority in expenditure be decided by the academic body itself.

Recent approval accorded to a scheme of promotion of university teachers by UGC is incongruous to the recent pronouncements of the UGC chief. Only recently UGC has approved automatic promotion from reader to professor and from lecturer to reader on the basis of length of service (13 years as lecturer and 10 years as reader) of an incumbent. This move was rejected by the Indian Science Congress (*Nature* 307, 100; 1984), but it is a worrying trend. Promotion should depend on open competition based strictly on merit without any bias tied to the length of service.

Finally, I am certain a great deal of practical wisdom is contained in the reports of various education commissions set up by the Government of India in recent years. The reports of the Radhakrishnan, Mudaliar, Mootham, Sen and Kothari commissions are gathering dust on various governmental shelves. The UGC chief could render an invaluable service by implementing relevant recommendations from each one of these reports, which can be implemented at the UGC level. Such actions would strengthen moves to persuade government machinery to take effective measures to save universities from academic deprivation. Genuine concerted efforts could turn a demoralized academic community into a professional force committed to seek and advance frontiers of knowledge and able to contribute its fair share in stimulating intellectual growth, nationally and internationally.

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Perils of too much disclosure

The New England Journal of Medicine would have authors disclose commercial interests in their articles. The American Institute of Physics will tolerate prior disclosure. Why?

WALL Street in the past year has been seriously damaged not by the unpredictability of the markets (from which it usually manages to profit) but in its reputation. A few months ago, it was alleged that an employee of the *Wall Street Journal*, a newspaper highly respected by the financial community, had been helping his friends to play the markets armed with the knowledge of what was about to appear in print.

Soon afterwards, an Arizona academic appeared on a television network, bad-mouthing G.D. Searle's new synthetic sweetener — and afterwards confessed that he had been selling Searle stock short in the expectation that its price would fall (which, surprisingly, it did not). Both tales are illustrations of what Wall Street and the other financial markets call "insider trading" — the exploitation of private knowledge by a few favoured people to make profits out of the ignorance of others. Their peculiar difficulty is that, in each case, the culprits are people not strictly subject to the regulations that govern the behaviour of recognized market makers.

For the financial markets and for governments, it has become a kind of nightmare to know how outsiders with inside knowledge should be prevented from cheating others. The agony seems now to have afflicted the *New England Journal of Medicine*, if anything even more highly respected than the *Wall Street Journal*. (At least, it does not fire off unsubstantiated charges that the Soviet Union is using genetic manipulation to manufacture biological weapons, as the *Wall Street Journal*, on the basis of a flimsy knowledge of what a gene is like, has recently been doing.) Last month, the *New England Journal*, in an article by its editor Dr Arnold J. Relman, raised the question whether the commercialization of medical research has not now become so "rampant" that those who publish scientific articles in the technical literature, but who also have interests in commercial companies, may suffer intolerable conflicts of interest (*New Engl. J. Med.* 310, 1182; 1984).

The question is interesting and important. That biomedical research has become uncomfortably commercial is widely accepted, even by some of those who hold this to be unavoidable or even desirable, for the sake of the rapid development of a new technology. That the publication of scientific articles can sometimes

affect the prices of particular stocks is also undisputed; the most obvious effects are those which follow the appearance of disappointing (negative) reports of trials with new drugs, but the phenomenon is not new. So how can journals ensure that they are not being misused by their contributors?

The *New England Journal's* suggestion, now opened for public discussion before being implemented as editorial policy, is that all contributors should declare direct commercial interests within what they seek to publish. An employee of, or one who has been helped financially by, an interested company should say so openly, but researchers who act as consultants for a company should make a declaration to the editor, who may afterwards ask that some of this information should be disclosed. On the face of things, this sounds sensible enough, entirely consistent with the contemporary fashion for publishing one's latest tax return before running for public office. But is the procedure necessary? And would it deal with the problem identified?

Especially since the latest surge of interest in biotechnology, most journals have found that full-fledged companies active in the field have been eager that their employees should clearly acknowledge who pays their salary. The declared motive is that the companies shall be better placed to recruit in the science labour market, but no doubt it helps on Wall Street that potential stockholders know the company is not asleep. The *New England Journal's* remedy would not change this state of affairs, but reinforce it. Practice suggests, however, that fast-moving corporations rely more on secrecy than on patent protection, with the result that the publication of important research is often delayed and, then, muffled.

In this climate, the most likely conflicts of interest are not those of contributors turned inside-traders but the following: members of the editorial staffs of journals may improperly use what they know about material not yet published, while contributors may tell lies in the interests of some secret sponsor. Fortunately, most journals are all too well aware of the dangers of even minor unauthorized disclosures. (*Nature* is still bruised by the angry withdrawal of an important article through the innocent transmittal of the report of a referee whose interpretation of the data was more interesting than the author's.) But the opportunities for financial gain are novel, and should be inhibited.

Contributors who may be tempted to shade the truth for hidden reasons are potentially a more serious problem. The *New England Journal's* recipe would help if only it could be enforced. The trouble is that those who may be the most tempted are the least likely voluntarily to disclose their interests. And the ultimate deterrent will be what it has always been — that those who shade the truth are eventually found out, and then damaged in reputation more seriously than their pockets can have benefited.

So, for the time being, *Nature* will not be following the policy with which the *New England Journal* is flirting, believing that present practices are most often sufficient to guard against abuse — and that the exceptions are those least easily dealt with by formal rules. Nor, for that matter, will *Nature* be following the new editorial policy announced by the American Physical Society (see *Physical Review Letters*, 11 June). On a quite different issue, that of the prior disclosure of the content of a scientific article, the society now says that it will not in future regard prior publication of the substance of an article in popular newspapers or magazines "with large circulations outside the physics community" as a bar to publication in its journals. It is shocking that this announcement should appear in the journal which, in the late Professor S.A. Goudsmit's time, most stoutly resisted the preference of many physicists for unrefereed publication on the front page of the *New York Times*.

The American Physical Society clearly does not appreciate what trouble this ill-thought policy will cause. Perhaps physicists are by now so dependent on the pernicious preprint system that they think their journals are archival from the start. But this new recipe is as neat a device as there could be for ensuring that important work is misrepresented to the public — one newspaper may sensationalize a hearsay account, the others will ignore the event for fear of seeming scooped. Even in the scientific press, broken embargoes may have the same disastrous consequences. This, in retrospect, is what may be made of the pallid account, in a recent issue of the journal *Science* (reference withheld), of a group of papers to be published in *Cell* and *Nature* in the next few weeks, and which may be the most important discovery so far this year. Thus will other tales of discovery be half-told if the American Institute of Physics is followed.

John Maddox

Physiology

Cardiac peptides and the control of sodium excretion

from G. A. Sagnella and G. A. MacGregor

PHYSIOLOGICAL experiments have repeatedly suggested the existence of natriuretic substances that increase the excretion of sodium from the kidney, probably by inhibiting the reabsorption of sodium along the renal tubule¹. These factors are of particular interest partly because the amount of sodium in the body's extracellular fluid regulates its volume, and partly because of suggestive evidence of a link between the kidney's ability to excrete sodium and essential hypertension¹, a major risk factor for cardiovascular disease².

It has recently become clear that there are at least two distinct types of natriuretic substances. One, probably produced by the hypothalamic region of the brain, is an inhibitor of ($\text{Na}^+ + \text{K}^+$) ATPase, the enzyme which transports sodium across membranes, and might be a contributory factor to a rise in blood pressure¹. The other, comprising peptides that have been isolated from the atria of the heart, has recently become the focus of attention for numerous laboratories throughout the world, although its physiological significance remains tantalizingly uncertain. The occasion for the sudden attention to the atrial peptides is their recent characterization and synthesis; moreover, as described on pages 717 to 726 of this issue of *Nature*, their precursor has now

been identified and its sequence deduced.

Both neuronal and humoral mechanisms have been suggested³ for the increase in urinary outflow that follows stimulation of atrial 'stretch' receptors⁴. Speculation that a novel endocrine system might be involved was stimulated by the observation that intravenous injection of rats with atrial, but not ventricular, extracts led to a striking natriuretic and diuretic response⁵. Moreover, the natriuretic activity is associated with endocrine-like secretory granules of the atrial cells^{6,7}, and the number of granules changes during water deprivation⁸.

Early work suggested that the active material was a peptide⁹⁻¹² and, more recently, it was shown to be a mixture of peptides of different molecular sizes which are not only natriuretic but can also relax the smooth muscle of vascular tissue¹³, causing vasodilation. A number of peptides — variously named atriopeptins, auriculins, cardionatrin and atrial natriuretic factors — have recently been purified and sequenced¹⁴⁻²⁰; their structures are summarized in Fig.1. There are two striking conclusions. First, all the rat peptides seem to be derived from a common precursor, as suggested by earlier indirect evidence⁹⁻¹² and consistent with the isolation of a 73 amino acid peptide containing the atrial natriuretic sequence in its

carboxy-terminal region²¹. Second, the one human peptide so far sequenced differs from the rat peptide by only one amino acid. All the peptides require an intact disulphide bridge for biological activity.

The suggestion of a common precursor for the peptides is now borne out by the sequences of precursor cDNAs of both rat and human atrial peptides reported by three groups in this issue of *Nature*. Both Yamanaka *et al.*²² and Maki *et al.*²³ have cloned cDNA of the atrial natriuretic factor derived from rat atrial mRNA, and have determined its nucleotide sequence and deduced an amino acid sequence. The precursor is 152 amino acids long and contains in its carboxy-terminal region the amino acid sequences of all the atrial peptides reported to date (Fig.1). It also contains a likely signal sequence with a high content of hydrophobic amino acids in the N-terminal region, which is a characteristic feature of precursors of secretory proteins²⁴. A remarkably similar sequence is reported by Oikawa *et al.*²⁵ for the cDNA encoding a precursor of the human atrial natriuretic peptide. (The deduced sequence contains not only the human atrial natriuretic peptide α -hANP, but also γ -hANP, a 126 amino acid peptide with the α -hANP sequence at its carboxy-terminus and which Oikawa *et al.* have identified in human atrial tissue — see Fig.1). Like the rat precursor, the human precursor contains a putative signal peptide sequence at its amino-terminus. There are some differences however — in particular, the Arg-Arg dipeptide following the atrial peptide sequence in the rat precursor is lacking in the human molecule. This may reflect differences in post-translational activation mechanisms.

The physiological function and importance of the atrial peptides have yet to be defined. Clearly, the fact that they have potent natriuretic and diuretic actions on the kidney as well as a vasodilatory effect raises the possibility of a role in the regulation of body fluids and blood pressure. Moreover, the peptides antagonize the vasopressor actions of the hormones noradrenaline¹⁷ and angiotensin II¹⁶. Although limited, the available evidence is compatible with a hypothetical scheme in which an increase in extracellular fluid volume may serve as a trigger for release of atrial peptides into the general circulation (Fig.2); obviously, a number of questions remain to be answered.

How these peptides relax vascular smooth muscle and increase sodium excretion is not yet clear. They do not seem to act by inhibiting the ($\text{Na}^+ + \text{K}^+$) ATPase¹², and there is conflicting evidence as to their effect on the glomerular filtration rate — atrial extracts have no marked effect on it²⁶, whereas synthetic atrial natriuretic factor increases it slightly¹⁶. Atlas *et al.* suggest that the natriuretic effect results from the vasodilatory action of the peptide and a shift of blood flow to the medullary region of the kidney¹⁶.

The characterization of the peptides at

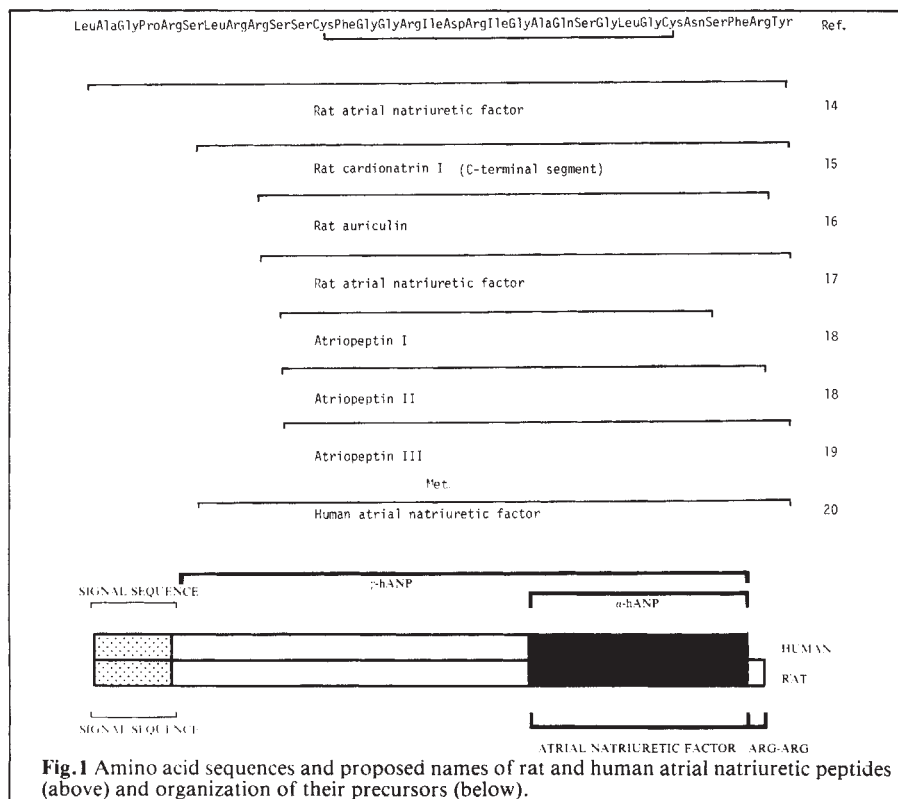
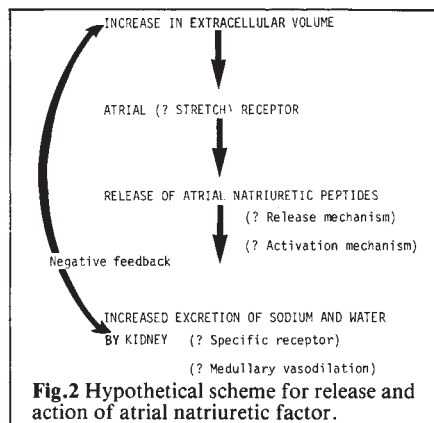


Fig.1 Amino acid sequences and proposed names of rat and human atrial natriuretic peptides (above) and organization of their precursors (below).



the molecular level and their subsequent synthesis provides a basis to investigate their mechanisms of action at the physiological and biochemical levels. There will undoubtedly be rapid progress in establishing radioimmunoassays to detect the presence of the peptides in the circulation and, if they are present, to test whether the circulating levels change with alteration of extracellular volume.

Clearly the potential roles of the peptides in disorders of electrolyte balance and in blood-pressure regulation remain to be clarified. For example, might Bartter's syndrome reflect excess atrial natriuretic factor and might hyporeninaemic-hypoaldosteronism be associated with a lack of it?

Finally, the relation of the atrial natriuretic peptides to other postulated, but as yet uncharacterized, natriuretic agents remains to be clarified. Teleologically, the existence of two natriuretic substances, one with vasopressor and the other with vasorelaxant properties, could allow the integrated control of both electrolyte and blood pressure homeostasis. □

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Optical physics

Generation of the shortest ever optical pulses

from Peter Knight

How many optical cycles do you need in a pulse for it still to be called a light wave? Ippen, Fujimoto and Weiner at MIT have just reported the generation and measurement of 16 femtosecond (1.6×10^{-14} s) optical pulses of centre wavelength 620 nm which contain just eight optical cycles (*Appl. Phys. Lett.* **44**, 832; 1984). They also report that they can see the colour of the light change as their high-repetition-rate laser apparatus compresses the optical pulses. These are the shortest light pulses ever generated and, since a molecule takes about 10 fs to vibrate, they open a new time-window in the study of ultrafast processes.

The generation of picosecond optical pulses has become fairly straightforward with the use of laser mode-locking. Because laser action is encouraged over as wide a frequency band as possible, many laser modes will oscillate at once. Mode-locking gives each mode a common phase so that constructive and destructive interference among the different waves leads to a regular train of pulses. Each of these pulses has a temporal width inversely proportional to the number of modes participating and thus to the laser gain bandwidth. For this reason a dye laser which can be excited over a wide frequency range can generate extremely short pulses. For example, a colliding-pulse ring geometry, developed at Bell Labs and now used by Ippen *et al.*, can generate reliable pulses of less than 100 fs duration.

The study of ultrafast phenomena requires even these short pulses to be further compressed in time. To achieve that degree of compression, Ippen *et al.* at MIT (and other groups in Bell Labs, IBM and Rochester) have stretched out the pulse frequency spectrum even more by 'chirping' the light so that the carrier frequency is swept. When this is followed by a dispersive element, the blue-shifted training edge catches up with the red-shifted leading edge and compresses the pulse.

Chirping can be produced by the nonlinear propagation of intense laser light in a liquid such as carbon disulphide. The intensity-dependent alterations in the refractive index of the liquid cause self-phase-modulation of the light. This method, proposed by Giordmaine at Bell Labs, has proved troublesome. The optical pulses can be chirped, but not in a reproducible or stable manner.

Nonlinear opticians have turned to single-mode glass fibres to produce stable uniform chirping. Light from a mode-locked laser can be focused into the small-diameter (about 10^{-3} cm) core of the fibre

to give internal intensities large enough for nonlinear effects to be important. The nonlinear refractive index produces a phase-shift which depends on the instantaneous intensity. The variable phase-shift increases until the peak of the pulse arrives and then it diminishes. The instantaneous frequency shift is given by the time-derivative of the phase shift, so that frequencies in the leading part of the pulse are lowered while those in the trailing edge are raised. The size of this frequency chirp increases directly with the length of the fibre. This chirping, combined with dispersion in the fibre, is the basis of the 'soliton laser' developed by Mollenauer and Stolen at Bell Labs (*Opt. Lett.* **9**, 13; 1984). The spectrally stretched pulse, on emerging from the glass fibre, can be compressed in time using a dispersive grating pair technique which allows the blue-shifted trailing edge of the optical pulse to catch up with the red-shifted leading edge.

Ippen's new experiment uses a colliding-pulse, mode-locked, dye laser to produce a train of pulses each of 65 fs duration. Single pulses are selected and amplified to initial energies of 5 μ J with a repetition rate of 10 Hz, and then spatially filtered, collimated and focused into an 8 mm long polarization-preserving fibre, which broadens the spectrum of pulse by a factor of about four by nonlinear chirping. This is followed by a grating pair to compress the pulse to its final length of eight optical cycles. The pulses are detected by a second-harmonic auto-correlator which determines temporal length by measuring the spatial extent of the pulse of light.

In a femtosecond, light travels one-third of a micrometre, so femtosecond pulses can be used to study a whole range of ultrafast processes which, until now, have been unresolvable. In condensed matter physics, such pulses can be used to study transport processes in semiconductors, non-radiative relaxation, and molecular vibration and re-orientation. In photochemistry and biology, direct observations should be possible of energy dissipation, transport and dephasing. The generation of femtosecond pulses has stretched nonlinear optics to the limit. With only eight cycles left in a pulse of visible light, any improvement would seem to demand a shift to the ultraviolet. Meanwhile, the new results of Ippen *et al.* will be eagerly applied to a wide range of transient phenomena. □

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Microbiology

Proteins that bind the β -lactam antibiotics

from A.F.W. Coulson

A MEETING that is concerned more with the enzymes that form the targets for the β -lactam antibiotics than with the β -lactamases that destroy them, would not sensibly be called a ' β -lactamase workshop'. But the name was coined in 1975 for the first of the workshops; by the third*, held recently, the focus had changed.

The target enzymes can be identified in whole cells by their binding to ^{14}C -penicillin G. In *Escherichia coli* there are two classes of penicillin-binding protein (PBP). The low-molecular-weight proteins, PBP-4, -5 and -6, are not essential to the organism, are DD-carboxypeptidases and are believed to regulate the degree of cross-linking of the cell wall. The high-molecular-weight PBP-1a, -1b, -2 and -3 have already been shown to be essential to *E. coli*; real progress in understanding the behaviour of these membrane-bound proteins is now becoming possible through the cloning and sequencing of their genes.

The sequence of the PBP-3 gene, representing a protein of 588 amino acids, was reported a year ago by Y. Hirota (National Institute of Genetics, Mishima). PBP-3 displays both transpeptidase and transglycosylase activities, and the latter is not inhibited by penicillins. B. Spratt (University of Sussex) presented evidence that the transpeptidase activity is associated with the carboxy-terminal half of the molecule, whereas the amino-terminal half contains the transglycosylase activity.

J. Broome-Smith (University of Sussex) has sequenced the genes for PBP-1A, PBP-1B and PBP-5. 1A and 1B are highly homologous; otherwise the only extensive sequence similarities in this group occur in the region of the tetrapeptide -Ser-X1-X2-Lys-. Tetrapeptides of the same form also occur in low-molecular-weight DD-carboxypeptidases and in several examples of each of two types of β -lactamase. For β -lactamase it has been very thoroughly established that the serine residue is part of the active site, and is acylated by a β -lactam in the course of its hydrolysis. Other similarities in the sequences of these three groups of enzymes are also concentrated in the neighbourhood of the tetrapeptide sequence. It is clearly very tempting to suppose both that the serine residue is part of an active site with a similar role in the mechanisms of all the enzymes, and that all the genes are homologous, at least in parts.

A highlight of the meeting was the presentation of the first high-resolution X-ray crystal structure of one of the proteins that interact with penicillins: the

2.8Å structure of a *Streptomyces* DD-carboxypeptidase (J. Kelly, University of Connecticut). The enzyme contains two regions of secondary structure — a six-strand β -sheet and a group of five stacked α -helices. To add to the progress that has been made in the last four years in extending the sequence-based classification of β -lactamases, R. Ambler (University of Edinburgh) reported a partial sequence of the Zn-containing β -II enzyme from *Bacillus cereus*, still the only member of class B. And J.O. Lampen (Rutgers University) presented a partial sequence of its gene.

The most interesting mechanistic study was that described by R.F. Pratt (Wesleyan

University, Connecticut) who showed that acyclic esters of the form $\text{Ar.CH}_2\text{.CO.NH.CH}_2\text{.CO}_2\text{R}$ are hydrolysed by β -lactamases — the first non- β -lactam substrates to be discovered. Transpeptidation can occur from these substrates to suitable amines, which implies that acyclic amides must also be substrates (J.R. Knowles, Harvard University).

The induction of penicillinase in bacilli has unusual features which have made it a long-standing problem, a solution to which may be at hand now that clones of the entire penicillinase operons are available (Lampen). It seems clear that the main oddities in the induction arise because the inducer modifies the cell wall giving rise to a 'second messenger' within the cell; the genetic control itself may be entirely conventional. The nature of the modification to the cell wall remains mysterious, particularly since it can be brought about by a variety of chemically very different agents.

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Astronomy

Quadrantid meteors

from David W. Hughes

It is none too easy to gather data on the Quadrantid meteoroid stream. January 3 or 4, depending on our phase in the leap-year cycle, is the only day of the year on which the Earth intersects the stream; the point on the celestial sphere from which the shooting stars appear to originate has a declination of $+48.5^\circ$, so Quadrantids are best seen from high latitudes in the Northern Hemisphere where winter makes visual and photographic conditions seldom satisfactory; and at least one year in four is hampered by moonlight. Moreover, the Quadrantid stream is very narrow, the time between half-maximum flux rates being about 12 hours, and if the maximum occurs when the radiant is low in the sky, the proportion of the flux that can be observed by any method is much reduced. This factor limits optimum observations of the shower to one year in every four.

Radar methods can overcome two of the problems, permitting the observation of meteors in daylight and through clouds. They, however, suffer from their own kind of 'bad weather', because radio propagation conditions in the ionosphere can vary considerably as a function of solar cycle. Another problem with radar is the identification of the shower meteors. This is usually accomplished simply by subtracting an assumed background rate from the total observed rate.

One goal of meteor stream investigations is to unravel the disintegration process of the parent comet and the subsequent evolution of the meteoroid stream. A knowledge of the particle number density

across the stream and around the stream orbit, and the way both of these vary as a function of particle size, would be very useful in that context. Hence the value of the determination by B.A. McIntosh, of the Herzberg Institute of Astrophysics, Ottawa, Canada, and M. Šimek, of the Astronomical Institute, Ondřejov, Czechoslovakia, of the long-term average cross-section of the Quadrantid meteoroid stream by analysing radar data obtained over nearly a quarter of a century (*Bull. astr. Inst. Czechoslovakia* 35, 14; 1984).

The Ondřejov and Ottawa radars are both pulsed systems and are similar in operation. Typical parameters are pulse length $10\mu\text{s}$, peak power 25 kW, repetition frequency 500 Hz. The antenna is fixed at an elevation of 45° and meteors within a line-of-sight distance of 400 km are recorded. Meteors are sorted into three duration classes, equivalent to median magnitudes of $+6$, $+1.6$ and -1.0 .

The practice of adding one year's data to the next is common with Quadrantid researchers but McIntosh and Šimek warn that it is not without problems. These become obvious when the combined data for the time interval 1958–81 are compared with the well-observed year of 1979. There are three basic numerical values which represent any flux curve: the time of maximum flux, the rate of rise and the rate of decay. The last two can be combined to give a stream width which is usually defined as the change of solar longitude that occurs during the time when the stream is more active than half its maximum activity. The

* β -Lactamase Workshop, Holy Island, Northumbria, 4–6 April.

authors noticed that the stream width from the combined data was twice that of 1979. The cause, they say, is the variation in the position of maximum, which can change by as much as 0.5° in solar longitude from year to year. So the annulus of Quadrantid meteoroids in the Solar System is not perfectly elliptical and deviations from this ellipse, at the descending node, can be as much as 7×10^5 km.

Contrary to the findings of many researchers, McIntosh and Šimek found that the maximum flux of bright meteors did not inevitably occur after the maximum flux of faint ones; in certain years the bright meteors preceded the faint ones. For all three meteor magnitudes the rate of rise of the flux to the maximum was slower than the subsequent fall. It was found, however, that the asymmetry increased as one considered brighter meteors. The theory of meteor stream production predicts that width should decrease as a function of meteor brightness. The authors found that the $+6.0$ and -1.0 magnitude meteors obeyed this rule but that the $+1.6$ magnitude meteors had a wider flux curve than either of the others.

In conclusion, it is clear that the Quadrantid shower, currently both one of the most active showers and the narrowest, has many peculiarities. These can, in the main, be blamed on Jupiter. The ascending node of the Quadrantid orbit occurs very close to Jupiter's orbit. Also the mean period of the Quadrantids is approximately half Jupiter's orbital period. This causes the nodes of the Quadrantid orbit to regress by about 0.4° per century, leading to mass sorting in the shower (smaller meteoroids usually peaking earlier than larger ones) and introducing short-term resonance fluctuations which again have a size-dependent effect.

Jovian perturbation is very selective. The small sector of the stream that is in the vicinity of Jupiter every 11.86 years will be shifted by larger amounts than the rest of the stream. The velocities of the perturbed meteoroids will be changed slightly and they will redistribute themselves around the orbit, eventually forming a low-density tail preceding the main body of the stream. McIntosh and Šimek conclude that the degree of asymmetry so produced is to some extent a measure of the age of the stream.

This leads us to one of the main unsolved questions concerning meteor streams. How old are they? We know that the Quadrantids are old enough for the meteoroids to have spread out fairly evenly around the orbit and for the parent comet to have completely disappeared. But the stream is still young enough to retain the record of slowness. If only we knew its age our ideas about stream evolution could be placed on a much firmer footing. □

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Molecular biology

DNA gyrations in reverse

from James C. Wang

In the well-known double-helix structure of DNA, the two strands revolve around each other. The topological problem of separating the parental strands during replication, particularly when the double-stranded DNA is in the form of a ring and thus the complementary strands are two intertwined single-stranded rings, was recognized more than two decades ago^{1,2}. The enzymes capable of solving such topological problems, DNA topoisomerases, are ubiquitous. They catalyse the transient breakage of the DNA backbone and the passage of DNA strands through the transient breaks³⁻⁵ in two mechanistically distinct ways. The type I enzymes break and rejoin one strand at a time and catalyse the crossing of one strand through another; the type II enzymes break and rejoin a pair of strands in concert and catalyse the crossing of a double-stranded DNA segment through a double-stranded break. It is not surprising that both types of activities are found in an archaebacterium *Sulfolobus*, as reported by Kikuchi and Asai on page 677 of this issue of *Nature*; what is amazing, however, is that *Sulfolobus* also contains an entirely new subclass of type II topoisomerase⁶.

Until now, two subclasses of type II topoisomerases had been found in eubacteria and eukaryotes. Bacterial gyrases, which fall into one subclass, are characterized by their ability to reduce the 'linking number' of a double-stranded DNA ring. The term 'linking number' is a topological quantity defined as the number of times one strand revolves around the other when the duplex ring is laid in a plane. If a DNA ring laid in a plane assumes the B-helical form, 10.5 base pairs make a complete helical turn and the linking number is the total number of base pairs divided by 10.5. When a duplex DNA ring is incubated with gyrase and ATP *in vitro*, the linking number is reduced by as much as 10 per cent below that of the most stable B-DNA structure. Gyrase achieves this energetically unfavourable reduction in linking number, termed 'negative supercoiling', by coupling DNA supercoiling to ATP hydrolysis.

The other subclass of type II topoisomerase includes those found in eukaryotes and phage T4 topoisomerase. These enzymes, though ATP-dependent, can only break and rejoin DNA to allow the passage of strands in such a way that the linking number of a duplex DNA ring 'relaxes' towards that of the most stable DNA structure, whatever its initial linking number; in this process, the chemical energy of ATP hydrolysis is not harvested by the DNA.

By contrast, the type II topoisomerase

found by Kikuchi and Asai in *Sulfolobus* increases the linking number above that of the most stable DNA structure by coupling ATP hydrolysis to the positive supercoiling of DNA. 'Reverse gyrase', as Kikuchi and Asai call their enzyme, thus becomes the founder member of a third subclass of type II topoisomerase, and an unexpected one, because no positively supercoiled DNA has ever been isolated from cells in physiological conditions.

Since all three subclasses are ATP-dependent and catalyse the passage of one double-stranded DNA segment through another, their differences lie in the directionality of duplex cross-over. Strand passage catalysed by the gyrase and reverse gyrase is directional, though opposite in sign, whereas strand passage catalysed by the other subclass lacks directionality and the DNA relaxes into a thermodynamic valley.

The discovery of reverse gyrase raises a number of questions. First, is it a distinct enzyme rather than an altered form of a gyrase of the same organism that has somehow changed its direction of strand passage? Mechanistically, it is an intriguing question whether directionality can be altered by changing the quaternary structure of an enzyme or by modifying its subunits. The scanty information that is available comes from studies with *Escherichia coli* gyrase. In the absence of ATP, gyrase can relax negatively supercoiled but not positively supercoiled DNA. However, a complex containing the A subunit of gyrase and what is probably a proteolytic fragment of the B subunit of gyrase, can relax both negatively and positively supercoiled DNA^{7,8}. Moreover, gyrase is stimulated to relax positively supercoiled DNA by the binding of the β, γ -imido-analogue of ATP⁹. Whereas such experiments show that it is possible to nullify the normal bias in the directionality of strand passage (relaxation of negatively supercoiled DNA) by the modification of a subunit of gyrase, it is more difficult to envision how strand passage could be switched from one direction to another by subunit modifications. The total lack of directionality in strand passage catalysed by phage T4 and eukaryotic type II topoisomerases is as intriguing as the directional strand passage catalysed by the other type II enzymes. In the case of the eukaryotic type II topoisomerases, the purified enzymes are homodimers. It is plausible that complexes between such enzymes and DNA have a twofold symmetry axis and that the symmetry abolishes any bias in the directionality of strand passage. The interesting question, then, is whether the binding of additional protein factors, or the

anchorage of the enzyme to a surface, can destroy such symmetry and make strand passage directional.

Is reverse gyrase unique to archaeobacteria? Even with hindsight, there has been no hint of such an enzyme in eukaryotes. Interestingly, however, positively supercoiled plasmid DNA has been isolated from *E. coli* following inhibition of its gyrase by novobiocin or oxolinic acid, although this can be attributed to the formation of a complex between DNA and drug-inactivated gyrase molecules¹⁰ — relaxation of a DNA when it is coiled around inactivated gyrase molecules is expected to increase its linking number¹¹. Furthermore, DNA gyrase is overproduced in cells treated with gyrase inhibitors¹², which might explain the slow time course for the formation of positively supercoiled *E. coli* plasmid DNA following the addition of gyrase inhibitors¹⁰. The observation that *E. coli* plasmid DNA isolated from temperature-sensitive gyrase mutants at non-permissive temperature is not positively supercoiled^{10,13,14} also argues against the existence of a reverse gyrase activity in *E. coli*. Nevertheless, the possibility of a reverse gyrase in eubacteria or eukaryotes cannot be discounted.

Why does *Sulfolobus* need a reverse gyrase in addition to its other topoisomerases? This is essentially the same question as why there are three topoisomerases in *E. coli*¹⁵. Whether some particular aspects of DNA in archaeobacteria require a reverse gyrase is unknown; even the state of DNA supercoiling in such organisms remains to be determined.

If there is one philosophical point to be gleaned from the coexistence of supercoiling and relaxing topoisomerases, it appears to be that nature not only solves the topological problems that come with intertwining of strands in the double-helix structure of DNA, but also seems to take full advantage of the topological properties of supercoiled DNA. At least in eubacteria, there is strong evidence that intracellular DNA is negatively supercoiled, that the

degree of unwinding influences many vital cellular processes including replication, transcription and certain site-specific recombinations³⁻⁵, and that multiple topoisomerases are, perhaps, needed to regulate the degree (see ref. 12 and refs therein). For eukaryotes, the first question is whether the evidence of their lacking supercoiling activity belies what actually occurs *in vivo*. Indirect evidence suggesting a supercoiled state in regions or subpopulations of intracellular DNA in eukaryotes

has been accumulating (see, for example, refs 16-18). With the recent identification of the gene encoding yeast type II topoisomerase^{19,20} and efforts of a number of laboratories in the search for other genes encoding eukaryotic DNA topoisomerases, powerful genetic tools can begin to complement the available biochemical approaches to answer this question. □

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Metallurgy

'Heavy' electrons in metals

from Tony Leggett

THE electrons in a typical metal interact quite strongly with the static crystalline lattice, with the vibrations of this lattice (phonons) and with one another. Many properties of nonmagnetic metals seem to be rather well explained, however, by a simple model, based on Landau's model of a Fermi liquid, in which the effects of these interactions are summarized by saying that the electrons behave as if they had an effective mass, m^* , which is different from their true mass, m , and a residual mutual attraction or repulsion. For almost all metals known until recently, the effective mass ratio m^*/m lies in the range 0.1-10 and the inter-electron interactions are not particularly strong. Great interest therefore attended the discovery of a class of metals which appear to be described by a Fermi-liquid picture but which have effective mass ratios of the order of 200 and, probably, correspondingly strong effective interactions. The interest was much increased when it was found that some of the metals of this class become superconducting at low temperatures, and is certain to be further heightened by the recent discovery that at least one of them, U_2Zn_{17} , undergoes a magnetic phase transition (*Phys. Rev. Lett.* **52**, 1551; 1984).

The systems in question are all compounds of a rare-earth element (usually cerium) or an actinide element (usually uranium) with one or more other metals: the most striking are $CeAl_3$, $CeCuSi_2$, UBe_{13} , UPt_3 and U_2Zn_{17} . From an atomic point of view, what they have in common is that the Ce or U atoms supply f-electrons, which are fairly tightly bound to the atom and would therefore be expected, in the solid, either to be strictly localized or to form a very narrow band, while the lighter elements supply p- or d-electrons which should be much more loosely bound; it seems probable that the electron states near the Fermi surface, which are expected to dominate the experimental behaviour, include both types. Probably the most striking experimental property shared by all these compounds is their enormous, low-temperature, electronic specific heats (C_{el}): while C_{el} is to

a first approximation proportional to T as in ordinary metals, the ratio is of the order of 1 J K^{-2} as against the usual value, $\sim 1\text{ mJ K}^{-2}$. If interpreted in the conventional Fermi-liquid picture, this yields effective masses of the order of 200 electron masses; correspondingly, the 'Fermi temperature' above which the electrons are no longer degenerate is of the order of 10 K, compared with $\sim 10^4\text{ K}$ for conventional metals. Although this huge effective mass could conceivably be due simply to the extreme narrowness of the band formed by the atomic f-electrons (the p- and d-electrons probably have much smaller effective masses and so contribute little to the specific heat), it seems very probable that it also has large contributions from strong electron-electron interactions.

While many of the experimental properties of the various compounds at temperatures above about 10 K are qualitatively similar, their low-temperature behaviour is strikingly different. $CeAl_3$ appears to undergo no phase transition of any kind down to the lowest temperatures reached ($\sim 0.02\text{ K}$); $CeCuSi_2$ has a superconducting transition which depends sensitively on such factors as preparation method and pressure; UBe_{13} and UPt_3 become superconducting at about 0.85 K and 0.54 K respectively; while, as the new paper shows, U_2Zn_{17} undergoes a phase transition at 9.7 K to what appears to be an antiferromagnetic state.

In trying to make sense of this bewildering variety of behaviour it is tempting to turn for guidance to the one extended laboratory system of fermions in which we know that inter-particle interactions are very important, namely liquid ^3He . Indeed, some experimental properties of the heavy-electron metals, particularly the specific heat at intermediate temperatures, show some striking similarities to those of ^3He . One important feature of the behaviour of ^3He that is often believed to be characteristic more generally of strongly interacting Fermi systems is the part played by long-lived spin fluctuations, which arise from the fact that the liquid is 'nearly ferromagnetic' (or 'nearly solid').

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Interestingly, when liquid ^3He becomes superfluid (the analogue of the superconducting transition in metals) the Cooper pairs form not, as in ordinary superconductors, with total spin S and angular momentum L equal to zero, but with $L = S = 1$. As a result, ^3He becomes an 'anisotropic superfluid' and has many unique properties. If, therefore, spin fluctuations are equally important in some or all of the heavy-electron metals, then it is tempting to speculate that these systems might become the first known superconductors to form Cooper pairs like those in ^3He ; that is, the first (genuinely) anisotropic superconductors.

In fact, some of the experimental properties of UPt_3 and UBe_{13} have already been interpreted as supporting this hypothesis; however, it may be some time before a really clear-cut demonstration one way or the other is available. In this situation, the discovery of an apparently antiferromagnetic phase transition in U_2Zn_{17} is particularly intriguing: it demonstrates clearly that magnetic interactions can be extremely important in heavy-electron metals, yet simultaneously indicates that in this case at least they are not, *prima facie*, of the kind that is likely to give rise to anisotropic superconductivity. Clearly we have a lot to learn about the subtle interplay of interactions in these fascinating systems. □

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Mammalian development

Methods and success of nuclear transplantation in mammals

from Anne McLaren

NATURE's own nuclear transplantation device, the fertilizing spermatozoon, provides cogent evidence for the role of the nucleus in heredity. The demonstration that cytoplasm too has a part to play had to await the exploitation by Danielli and his colleagues of a microsurgical technique of nuclear transplantation in *Amoeba*¹. Soon after, Briggs and King succeeded in transplanting nuclei from cells of frog larvae into enucleated frog eggs². Their initial results suggested that nuclei become restricted in potency as development proceeds; but in a long series of experiments Gurdon and his colleagues showed that cell differentiation does not necessarily result in irreversible nuclear changes³. Thus nuclei from differentiated intestinal cells of tadpoles injected into enucleated frog eggs can be reprogrammed by the egg cytoplasm so as to support the development of adult frogs⁴; however, while nuclei from cells of adult frogs can support the development of tadpoles, the tadpoles fail to metamorphose into adults^{5,6}. The widespread belief that clones of adult frogs have been produced by trans-

planting nuclei from adult tissue is thus fallacious.

Still more fallacious, of course, is the account of human cloning in Rorvik's *In His Image*⁷. Indeed, nuclear transplantation in mammals has proved very difficult to achieve. Since a mammalian egg is a thousandth or less of the volume of a frog egg, this is hardly surprising. Microsurgical techniques have been devised both to remove single pronuclei from mouse eggs⁸⁻¹⁰, giving haploid embryos, and to transplant embryonic nuclei into fertilized mouse eggs, giving a tetraploid chromosome complement¹¹. Both procedures are followed by cleavage to the blastocyst stage, but attempts to combine the two procedures by transplanting nuclei into mouse eggs from which both pronuclei have previously been removed invariably lead to disintegration of the eggs (J.A. Modlinski, personal communication). Attempts have also been made to introduce nuclei from eight-cell embryos into unfertilized rabbit eggs¹², either by microsurgery or by virus-mediated cell fusion, but the success rate is low, and no more than two to three cleavage divisions are ever observed. Virus-mediated fusion of somatic cells with mouse eggs has shown equally little promise^{13,14}.

The first report of mice born from nuclear transplantation came from Illmensee and Hoppe¹⁵. The trauma of two operations on the same egg was avoided by injecting donor nuclei into fertilized eggs and then immediately sucking out the two pronuclei of the recipient, using the same pipette. The donor nuclei were taken from blastocysts: if from inner cell mass cells, the recipient eggs sometimes developed to term but if from trophectoderm cells development did not proceed beyond mid-cleavage. A similar difference was reported for nuclei transplanted to non-enucleated fertilized eggs to produce tetraploid embryos¹⁶: eggs receiving inner cell mass nuclei developed into blastocysts, but those receiving trophectoderm nuclei blocked at the eight-cell stage.

The microsurgical technique of Illmensee and Hoppe has not yet been successfully carried out in any other laboratory, though it has been attempted by at least four groups, and probably more. In contrast, a technique devised by McGrath and Solter¹⁷ has already been used with success in our own laboratory¹⁸ and another¹⁹. Their technique avoids the necessity of penetrating the plasma membrane of either the donor or the recipient egg by sucking out the pronuclei surrounded by a piece of plasma mem-

100 years ago

WORK-MEASURING MACHINES

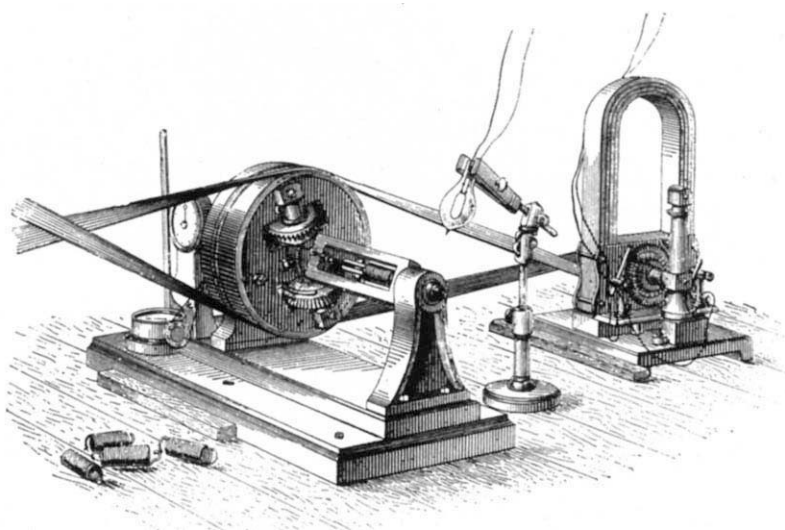
UNDER this title a little *brochure* has recently appeared from the pen of the Rev. F. J. Smith, B.A., of Taunton, in which work-measuring dynamometers, or *ergometers*, of various forms are described. Amongst these machines there are many devised by the author himself, and some of these are of considerable interest and much originality.

The leading feature of this instrument is the position of the spring in it. The axis of the spring

and of the shaft coincide; the result of this is that it is hardly at all affected by centrifugal force. When springs of slight pull are used and the ergometer is driven at a great velocity, the deformation is considerable, and would introduce considerable error in the result.

The deformation of the spring has been fully appreciated and therefore Rev. Smith has placed the spiral springs of his ergometer in cylindrical cases. When the spring is placed with its axis coincidental with that of the machine, no such error can be introduced and the friction of a spring against a case is avoided.

From *Nature* 30, 220, 3 July 1884.



Smith's Transmission Ergometer

brane — a karyoplast. The donor karyoplast, together with some Sendai virus to promote fusion, is injected under the zona pellucida of the enucleated recipient egg, with which it fuses. The yield of live mice is very high when fertilized eggs are used as donors, but no successful development beyond the blastocyst stage has so far been reported with donor nuclei from older embryos.

One application of the technique has been to exchange pronuclei between fertilized eggs of different strains, so as to investigate whether the basis for maternal effects transmitted via the egg resides in the nucleus or in the cytoplasm at the time of fertilization. In this way expression of the stage-specific embryonic antigen SSEA-3 has been shown to depend on the egg cytoplasm²⁰, while the lethal effect of the hairpin tail mutation when transmitted through the mother seems to be a property of the pronuclei²¹.

Another application has been to exchange pronuclei between fertilized and parthenogenetically activated mouse eggs in an attempt to understand the mysterious failure of parthenogenotes, whether haploid or diploid, to develop to term. When pronuclei from eggs parthenogenetically activated by alcohol treatment were transplanted by the McGrath and Solter technique into enucleated fertilized eggs, the embryos did not survive to term; but pronuclei from fertilized eggs, transplanted into parthenogenetically activated and then enucleated eggs, produced live mice¹⁸. This result suggests that the defect lies in the pronuclei, not in the cytoplasm. In contrast, when inner cell mass nuclei from parthenogenetic blastocysts were microsurgically transplanted into enucleated fertilized eggs, using as donors spontaneously activated LT/Sv embryos, Hoppe and Illmensee reported that they developed normally²².

Diploid parthenogenetic embryos are a special case of embryos with two maternal and no paternal genomes: such embryos can also be derived by removal of the paternal pronucleus, followed by doubling of the remaining maternal pronucleus. Such homozygous diploids, whether formed from the maternal or paternal pronucleus, have been reported to develop to term⁹, but attempts to confirm these results have not been successful^{18,23,24}.

Using the McGrath and Solter technique to exchange single pronuclei, it has recently been shown that normal development does not follow the addition of a second maternal pronucleus to a haploid parthenogenote, whereas the addition of a paternal pronucleus results in a viable embryo¹⁹. Also, while a paternal and a maternal pronucleus taken from fertilized eggs and recombined will support development, two paternal or two maternal pronuclei fail to do so²⁵. Unless some extrachromosomal component of the pronucleus plays a hitherto unsuspected role in development, these two pieces of work suggest that

normal development requires both a paternal and a maternal chromosome set.

Only when we understand the reasons for the developmental failure of certain categories of embryo will it be possible to assess the significance of these results as a whole. In attempting to resolve the apparently conflicting lines of evidence summarized above, certain points must be borne in mind. When pronuclei are sucked out with a fine pipette, some or all of the pronuclear membrane may remain behind even though the pronuclear contents are removed. When nuclei are transplanted, either by microinjection or cell fusion, some cytoplasm is also transferred. Eggs artificially activated by exposure to alcohol cannot necessarily be equated with spontaneously activated LT/Sv eggs. Finally, inner cell mass nuclei cannot be equated with pronuclei: it may be that by the blastocyst stage, developmental potential has become restricted; alternatively, some developmental block affecting the nuclei of parthenogenetic eggs may have been overcome by this stage.

A precedent is seen in the *o* maternal effect lethal of the axolotl. This involves a cytoplasmic effect such that eggs from homozygous females, that would otherwise die during gastrulation, can be rescued by injection of normal oocyte cytoplasm or nuclear sap at the one-cell stage²⁶. Transplantation of normal nuclei into enucleated *o/o* eggs is followed by normal development if the donor nuclei are taken from mid-blastula or later embryos, but not if they are taken from early blastulae, suggesting that some heritable change takes place in the axolotl nucleus during the blastula stage²⁷.

What of the future? Now that mouse strains carrying mitochondrial variants²⁸ are available, the use of recipient eggs carrying a mitochondrial marker will provide unequivocal evidence that a mouse is indeed derived from a nuclear transplant, just as the one-nucleolus marker provided similar certainty for amphibian nuclear transplantation³. The McGrath and Solter technique can be used to analyse any maternal effect exerted via the egg²⁹, and also perhaps to examine the roles of nucleus and cytoplasm in oocyte maturation. But only if it can be extended to use nuclei from later stages of development, or if the Illmensee and Hoppe nuclear transplantation technique can be successfully repeated, will the type of analysis pioneered in Amphibia also become possible in mammals. Does the developmental potency of nuclei become irreversibly restricted in the course of differentiation? Can somatic nuclei be reprogrammed, perhaps by transfer through several successive oocyte hosts? Can even adult nuclei be persuaded to revert to their ancestral totipotent condition? If not, why not? Perhaps irreversible gene rearrangements during somatic development, of the type undergone by immunoglobulin genes in the development of antibody-producing

B cells, are more common than we realize. Mammalian systems may throw light on these crucial questions, at present unanswered even in Amphibia.

The potential rewards in animal breeding are great, with the cloning of prize-winning animals as the ultimate goal. Unless it becomes possible to use adult nuclei for renucleation of enucleated eggs, it will not be possible to clone champions, as such. But if large numbers of genetically identical embryos could ever routinely be produced by breeders, using inner cell mass nuclei to renucleate eggs, most could be stored in frozen form while waiting to learn if a champion developed from one that was taken to term. If so, the frozen embryos could be thawed and transferred to foster mothers as required.

For human embryos, nuclear transplantation has a bad name because of the science fiction overtones of cloning; but for couples at risk of transmitting a genetic defect to their progeny, it could provide a method of screening that did not involve, as present methods do, termination of the affected pregnancies. If several eggs were recovered from a woman and renucleated with inner cell mass nuclei from one of her own embryos, a few could be frozen, while the genetically identical remainder were screened for a genetic defect, by chromosome analysis, DNA analysis or other biochemical methods. In this way it would be possible to assure the woman that any embryo transferred to her uterus from the frozen stock would be free of the screened-for genetic defect. □

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Direct land/sea correlations in the last interglacial complex

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Isotopic and pollen analyses of an eastern Atlantic core permit the first direct correlation of substages 5e and 5c of the marine climatostratigraphical system with the Eemian and St Germain I, respectively, of the classical Grande Pile pollen sequence (north-east France). The vegetational sequences during the warm intervals of the last interglacial complex (125,000–75,000 yr BP) indicate that climatic deterioration occurred late on the mid-European continent after the initiation of glaciation over continental masses in high latitudes.

IN the reconstruction of the global climatic patterns of the succession of glacial and interglacial periods of the Quaternary, the correlation of deep-sea records and of continental data is of the first importance. Because the $\delta^{18}\text{O}$ signal in continuously deposited deep-sea records is chiefly determined by global ice-volume^{1,2}, it is globally synchronous in the open oceans and thus provides a basic climatic stratigraphy for oceanic events.

Unfortunately, land-based records cannot be easily correlated with the oceanic records. First, there are several independent climatostratigraphical systems³ based on palynological data, studies of loess–soil succession and of the geomorphological and sedimentological features of terraces and moraines. Second, because many of the data extend beyond the ^{14}C time scale, their chronology is only poorly controlled. Finally, the discontinuity of continental records impedes inter-regional correlations. The result is that there is no reliable linkage between continental units and the now-standard oceanic ^{18}O succession^{3,4}.

This is true even for the last interglacial period (125,000–75,000 BP), which most closely resembles the present in climate and which might serve as a guide to future climatic conditions. Thus, in palaeoceanographic studies, oxygen isotopic data^{5–8}, changes in marine faunal assemblages^{9–12}, and carbonate curves¹³, show that the last interglacial interval^{15,7,9,14,15} (125,000–75,000 yr BP), which was the last globally warm period before the present, was characterized by a succession of three main warm phases separated by two cold phases. This palaeoclimatic history has been also recorded in ice cores^{16,17} and in the succession of coral terraces^{18,19}.

In the oxygen isotope stratigraphy, the last interglacial interval corresponds to Emiliani's stage 5 (ref. 5) in which the oldest subdivision (Shackleton's substage 5e; ref. 7) can be regarded as the warmest period of the whole interglacial complex and at least as warm as today. Fragmentary continental records have revealed that a climate as warm as the present one certainly occurred during the Eemian stage in northwestern Europe. On the basis of similarities between climatic conditions recorded in deep-sea cores and continental sequences, Shackleton⁷ first suggested that the northwestern European Eemian interglacial was the continental equivalent of substage 5e. But now it seems that in moraine systems there were three stratigraphically separate Eemian interglacials^{3,4}, and that numerous truncated Eemian deposits (including the type Eemian of the Netherlands) cannot be correlated with stage 5e. In fact, the best and most complete climatic succession through the last interglacial complex has been obtained later from the long continental sequences of Grande Pile (northeastern France)²⁰ and Tenagi Philipon (northern Greece)²¹. These sequences reveal that there was a succession of three warm intervals during the interglacial, as observed in the deep-sea record. Nevertheless, the striking resemblance between the climatic evolutions observed in both

land and sea sequences does not necessarily imply precise synchronism between the successive parallel climatic events. Indeed, discrepancies remain concerning the real correlation of French Eemian, Greek Pangaion and subsequent warm periods with the oceanic stratigraphical framework of the last interglacial complex^{20,22–25}.

In view of the difficulties which are encountered in continental sequences, marine palynology seems to offer the most valuable method for direct land/sea correlations. It has recently been shown that pollen and spores contained in deep-sea sediments neighbouring coasts may indeed reflect the adjacent continental vegetation^{26–28}. Moreover, marine palynological sequences without major lacunae can be directly correlated *in situ* with deep-sea isotope stratigraphy. Thus core records from the north-eastern Pacific²⁹ and Bay of Biscay³⁰ permit direct marine/continental correlation between events of the last deglaciation (20,000 yr BP–present). In the same manner, off the Washington–Oregon coast³¹ and in the Arabian Sea³², the correlation of the vegetational evolution of the last interglacial complex with the isotopic subdivisions of stage 5 has also been demonstrated.

By means of palynological studies from a deep-sea core located off Iberian coasts in the Atlantic ocean, I aim here:

- (1) To determine, for the first time, the vegetational and palaeoclimatic evolution of the adjacent southwestern European continent during the last interglacial complex.
- (2) To correlate continental events recorded in the present study, during isotopic substages 5e, 5d and 5c, with the lower part of the continuous palynological diagram from the Grande Pile peat-bog, which is today the nearest continental site to the deep-sea record studied.
- (3) Thence, to correlate directly the lower part of Grande Pile sequence with the standard isotope $\delta^{18}\text{O}$ stratigraphy, which has been established in the marine core.
- (4) To determine precisely the relation between the onset of ice growth phases in polar areas and the continental response in southwestern Europe to climatic changes, which occurred at the transition between warm isotopic substages 5e, 5c and the full glacial conditions of substages 5d and 5b.

Isotope and pollen records in core SU 8132

Core SU 8132 was recovered near the continental slope 100 km off the Iberian coast, near the Spanish–Portuguese border (42°06'N, 09°41'W, 2,280 m). As has already been shown in the Bay of Biscay²⁶, deep-sea sediments at the same distance from vegetated landscapes, contain numerous pollen and spores, the distribution of which reflects the regional vegetation of the adjacent continent.

Oxygen isotope records were made on the basis of analyses of the planktonic foraminifer *Globigerina bulloides* (> 250 μm fraction)². Because benthic $\delta^{18}\text{O}$ records are less influenced by

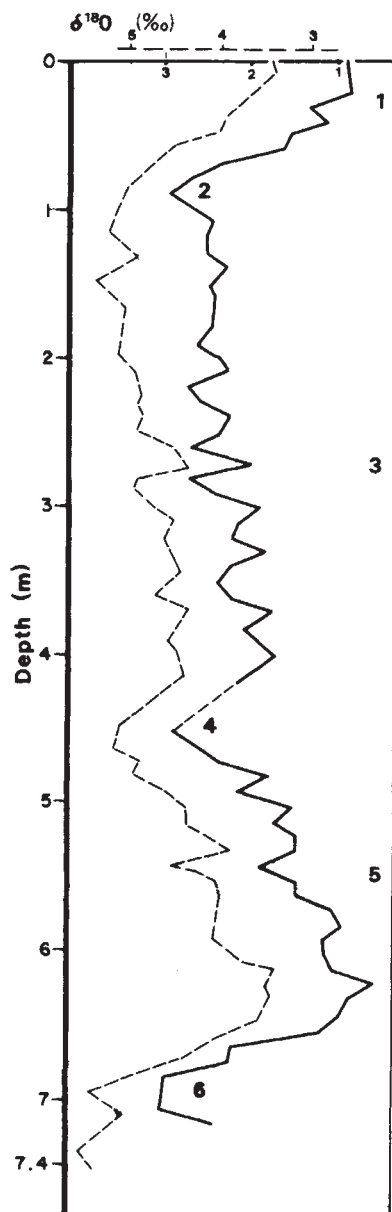


Fig. 1 Oxygen isotope, $\delta^{18}\text{O}$, record of planktonic foraminifer *Globigerina bulloides* (solid curve) and benthic foraminifers after normalization to *Uvigerina peregrina* (dotted curve) in core SU 8132, after J. C. Duplessy (personal communication).

local effects than planktonic ones³³, benthic foraminifera were also analysed to obtain a better isotope stratigraphical framework, primarily reflecting global ice volume evolution at high latitudes^{34,35}.

The $\delta^{18}\text{O}$ record (Fig. 1) shows that core SU 8132 represents a high sedimentation rate and gives a long continuous sequence with the classical development of Emiliani's stages⁵ from 6 to 1. Unfortunately, during stage 5, Shackleton's upper substages⁷ 5b and 5a are difficult to determine because of the low rate of sedimentation at that time, and/or the disturbed nature of the sediment. By contrast (Fig. 2), substage 5e is well developed from 680 to 580 cm, representing a high sedimentation rate for this period (100 mm kyr⁻¹). The glacial/interglacial change at the 6/5e boundary is marked by a major shift at 680 cm corresponding to Termination II of Broecker and Van Donk¹⁴. Substage 5c is also identifiable between the two major $\delta^{18}\text{O}$ increases at 540 cm (substage 5d) and 490 cm (lower boundary of 5b). There is another major $\delta^{18}\text{O}$ shift corresponding to the stage 4/stage 3 boundary at 420 cm.

In core SU 8132, palynological analysis has only been applied to the interval between stages 6 and 3. Samples were treated according to classical methods: HCl, HF and heavy liquor, and sieving on 15–20 μm mesh. Some 100–200 grains were counted (excluding *Pinus* (pine) pollen). To delete the overrepresentation of *Pinus* pollen grains that affects pollen profiles in marine sediments³⁶, percentage evaluations on Fig. 2 were based on the total pollen grains, including AP (arboreal pollen minus *Pinus* pollen), and NAP (non-arboreal pollen) and spores of ferns. The pollen diagram shows warm intervals in which temperate tree pollen dominates, alternating with cool intervals in which non-arboreal pollen is relatively more important.

At the end of stage 6, pollen spectra reflect an open cool vegetation (Graminae, Cyperaceae, Ericaceae, rare *Artemisia* and other Compositae) representing up to 95% of the basic sum. Arboreal pollen (except for *Pinus*) is very rare. At the boundary of stage 6/substage 5e, Termination II is very well marked by the immigration of *Betula* (birch) and the first appearance of *Quercus* (oak) (pollen zone 2). The early part of substage 5e is characterized by the maximum relative value of *Quercus* (pollen zone 3). Abundant *Quercus* is accompanied by a noticeable increase in *Carpinus* (hornbeam) (pollen zone 4), followed in the latter part of substage 5e by the well-marked development of *Abies* (fir) and a slight spread of *Betula* (pollen zone 5). At the 540-cm level, the pollen profile reflects the return of cold conditions with a major drop in the AP curve directly correlated with the cold event 5d (pollen zone 6). The sharp $\delta^{18}\text{O}$ decrease which followed (substage 5c) is characterized by the dominance of temperate tree pollen, with an early *Quercus*–*Carpinus* succession accompanied by a slight increase in *Abies* (pollen zones 7, 8 and 9).

Another short-lived disappearance of temperate forest occurs in pollen zone 10. Pollen zone 11 is characterized by a slight immigration of *Quercus*, but the landscape is dominated throughout by herbaceous plants. As has been previously noted, the sedimentary sequence of this part of core SU 8132 seems disturbed and, therefore, the vegetational record could be either discontinuous or mixed.

Pollen zones 12 and 13 at the top of the sequence show an alternation between assemblages without forest development, which corresponds to stage 4, and an increasing proportion of temperate trees (*Betula*, *Quercus*, *Abies*) correlated closely with the early part of stage 3.

Thus marine pollen analyses from core SU 8132, being directly correlated with oxygen isotope stratigraphy, show for the first time that during the early and middle part of stage 5, the palaeoclimatic evolution of the neighbouring western Iberia was characterized by a succession of two warm phases (5e = pollen zones 2–5; 5c = pollen zones 7–9) separated by an abrupt and very cold interval (5d = pollen zone 6).

Moreover, during the two older warm periods of stage 5 (5e and 5c) a logical succession is observed in the immigration of various temperate trees starting after the glacial episodes (6 and 5d)—early development of *Betula* soon replaced by the immigration of *Quercus*, then marked extension of *Carpinus* and a late increase in *Abies*. This vegetational evolution is in good agreement with the main criteria taken in continental pollen sequences to characterize, from a palynological point of view, a complete interglacial cycle^{25,37–40}. By comparison, the early part of stage 3, which is marked by the simultaneous immigration of *Betula*, *Quercus*, *Abies* and lack of *Carpinus*, must be interpreted as an interstadial period.

It can be seen that the pollen assemblages from substage 5e and the pollen assemblages from substage 5c are generally similar but 5e slightly differs from 5c in the more important role of *Abies* in the latter part of the episode.

Correlation with continental sequence

Many continental sequences covering the last interglacial climatic cycle in northwestern Europe have been studied by means of pollen analyses^{41–46}. They indicate that a warming period, for example, the classical Eemian^{47,48} in Holland, occurred

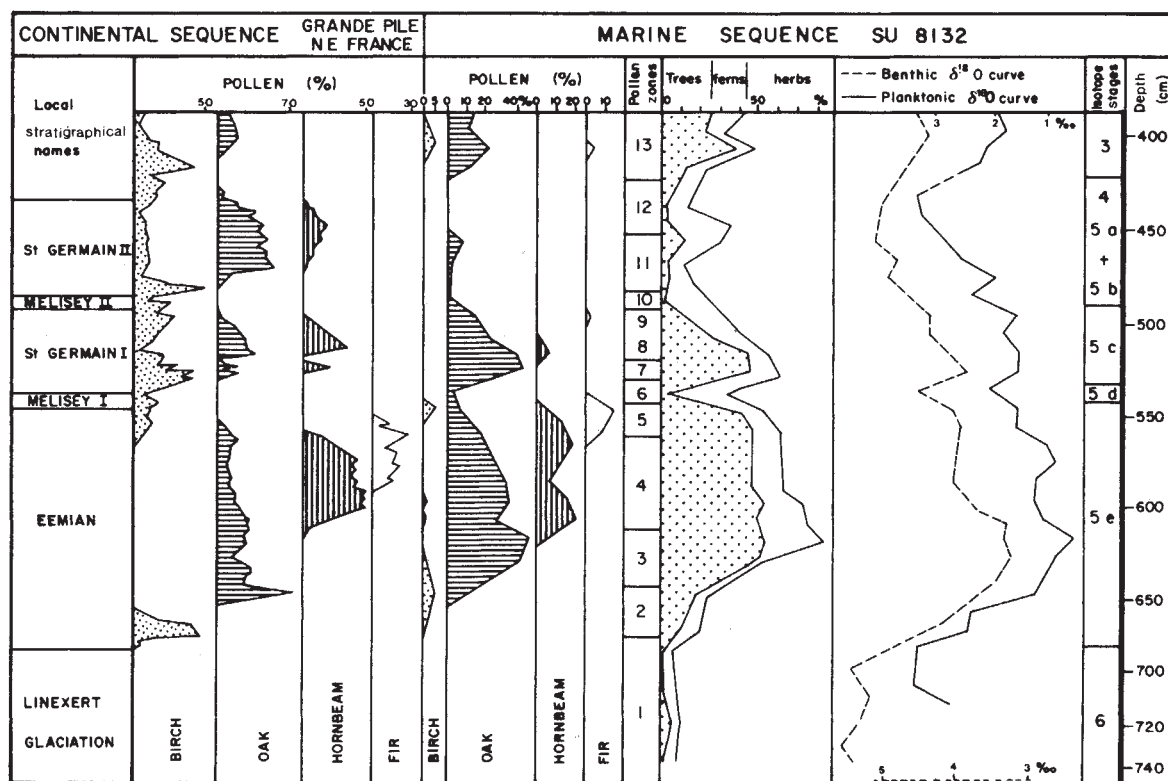


Fig. 2 Direct correlation of $\delta^{18}\text{O}$ stages with the continental sequence of Grande Pile by means of the marine palynological sequence of core SU 8132. Continental sequence after ref. 20. Benthic and planktonic $\delta^{18}\text{O}$ after J. C. Duplessy (personal communication). In the marine sequence, the relative frequency for pollen of tree species is expressed as a percentage of the total pollen + spores, excluding pine pollen.

below the last Weichselian glaciation. Unfortunately, few continental deposits apparently contain a continuous pollen record extending over the entire last interglacial complex. Lack of evidence for a complete record of subsequent climatic evolution up to the present interglacial, and absence of accurate absolute dating methods have often posed the problem of their real place in the interglacial/glacial succession of continental climato-stratigraphical systems^{3,4,49}. One of the best and most complete sequences known at present is from the Grande Pile in north-east France²⁰. Figure 2 shows that between the end of a long glacial period (? Saale, ? Warthe) and the beginning of the Würm glaciation, which is radiometrically dated at ~70,000 yr BP (ref. 50) there were three temperate intervals, namely Eemian, St Germain I and St Germain II, separated by two very cold phases, Melisey I and Melisey II (Fig. 2). Furthermore, the early warm period (Eemian) is characterized by the successive extension of the tree species *Betula*, *Quercus*, *Carpinus*, and *Abies*, very similar to that recognized in core SU 8132 during isotopic substage 5e. In the Grande Pile record St Germain I slightly differs from the Eemian mainly in the lack of *Abies*, in the same manner that the development of *Abies* in marine substage 5c is less important than in 5e. As in the marine record during 5d, palynological evidence from Grande Pile shows that Melisey I was an abrupt and very cold period which separated the two older warm intervals of the interglacial complex. However, some Eemian and St Germain I interglacial features do not occur in the deep-sea record: there is a lack of *Picea* (spruce) and *Corylus* (hazel) phases, and a lower species diversity of herbaceous taxa. These can be explained by taking into account the southerly position of core SU 8132, the selective effect of the regional (but not local) origin and marine transport processes of the marine pollen assemblage²⁶. Therefore, despite some slight differences, the marine pollen diagram exhibits the same climatic trends, and a similar general succession of vegetation landscapes as in north-east France and there now seems to be no doubt that Woillard's Eemian and St Germain I interglacial periods corre-

spond, in core SU 8132, to substages 5e and 5c respectively, and that Melisey I correlates closely with substage 5d.

Despite some controversies in earlier estimates^{15,51-53}, there is now general agreement on assigning dates of ~125,000 and 75,000 yr BP to Emiliani's stage 6/5 and 5/4 boundaries respectively⁵⁴⁻⁵⁶. Radiometric dating of coral terraces¹⁴, and $\delta^{18}\text{O}$ time scales determined by assuming a constant sedimentation rate, are so similar that a correlation of the three $\delta^{18}\text{O}$ minima 5e, 5c, 5a and high sea level stands on Barbados at about 125,000, 104,000 and 80,000 yr can be demonstrated¹⁵. Therefore, extrapolated ages for the two cold peaks (5d, 5b) can be estimated as 110,000 and 90,000 yr BP, respectively.

These peaks are synchronous within the mixing time of the world's ocean waters, and the marine palynological evidence of SU 8132 allows us to conclude that Woillard's Eemian and St Germain I interglacials are correlated with Barbados I and Barbados II, while the lower boundaries of Melisey I and II can be placed at ~115,000 and 95,000 yr BP respectively.

This direct correlation between deep-sea chronology and the lower part of the continental stratigraphical sequence from Grande Pile confirms and gives precision to the previously hypothetical correlations suggested by Woillard and Mook⁵⁰ for this interglacial complex. Conversely, the results of the present study invalidate correlations of the Eemian interglacial with the whole of stage 5 or with substages 5e, 5b, 5c combined, as suggested by Morner^{23,24}.

Continental vegetation and ice sheet growth

In core SU 8132, the oxygen isotopic curve based on benthic foraminifera shows that after a well marked minimum in the lower part of substages 5e and 5c, $\delta^{18}\text{O}$ values began to increase within the middle part of these two warm interglacials long before the onset of glacial substages 5d and 5b (Fig. 2). This would indicate that an early phase of ice growth took place in polar latitudes before the end of both Eemian and St Germain I time. From numerous North Atlantic cores, Ruddiman *et al.*⁵⁷

observed a similar trend across both 5e/5d and 5a/4 transitions.

Moreover, pollen data from core SU 8132 (Fig. 2) clearly indicate that the beginning of each ice growth (at 610 and 520 cm) is directly correlated with pollen zones 4 and 8 respectively, which are both characterized by the spreading of *Carpinus*. From pollen assemblages recorded in continental deposits during past interglacials it appears that the classic *Carpinus* phase is often marked by the presence of thermophilous plants such as *Ilex*, *Buxus*, *Hedera* and *Viscum*^{20,40,41,47,58}. Thus, it may be assumed that the *Quercus*–*Carpinus* vegetational succession does not indicate climatic deterioration but, on the contrary, general stability of warm temperatures during the climatic optimum of each interglacial period. According to Andersen⁵⁸ this classical succession of vegetation landscapes could be conditioned by soil evolution rather than by climatic change. In fact, from the SU 8132 sequence it appears that temperatures in southwestern Europe probably began to decrease only with the immigration of *Abies*, which occurred at the end of both interglacial intervals, but that full glacial conditions, marked by high amounts of herbaceous plants, did not start before major ice volumes had been accumulated in Arctic zones during substages 5d and 5b. All these data indicate that the initiation of Boreal ice caps, during the second half of the Eemian and St Germain I interglacials, did not correlate with a temperature decrease in southwestern Europe, probably because an ice cover was not yet established over Scandinavia.

This pattern agrees well with the evolution of palaeotemperatures in the northeastern Atlantic ocean at interglacial to glacial transitions during stage 5. By means of matched isotopic and marine faunal data, Ruddiman and McIntyre⁵⁹ pointed out the existence between 40° N and 60° N of warm, eastern North Atlantic sea-surface temperatures during the first half of rapid ice growth. They speculated that along with low summer insolation, which occurred at the 5e/5d and 5a/4 transitions, an 'interglacial' warm Atlantic Ocean might be a necessary condi-

tion for delivering moisture to high latitude areas, thereby leading to the initiation of extensive glaciation.

Moreover, according to Royer *et al.*⁶⁰, the cooling linked to the insolation change, which occurred around 115,000 yr BP, was not uniformly distributed over the northern high latitudes, but has a well defined maximum, combined with increased precipitation, over North America. These data suggest that initial high-latitude glaciation was established on the western side of the North Atlantic Ocean. In this context, evidence from the SU 8132 record indicates that the initiation of the Laurentide ice sheet did not cause a temperature decrease in southwestern Europe, and probably excluded the simultaneous inception of ice caps in Scandinavia. It is only later, at the end of the interglacial periods, that the disappearance of *Carpinus* forest and extension of *Abies* phases probably indicates the mid-European continental response to the onset of glaciation over Scandinavia.

Finally, the present study provides additional data on a parallel climatic evolution between the eastern North Atlantic Ocean and the adjacent European continent, and on the diachronous inception of ice caps on either side of the North Atlantic Ocean. This pattern agrees well with a drop in sea level during the Eemian while the contemporaneous climate in western Norway probably inhibited the growth of any large ice-masses in Scandinavia⁴⁶. A marked cooling of sea-surface temperatures, which occurred in the western North Atlantic and in the North Pacific⁶¹, while both the eastern North Atlantic and the adjacent European continent remained warm, could probably be regarded as a natural trend which might have later promoted the transition to full glacial conditions in the Northern Hemisphere.

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Reverse gyrase—a topoisomerase which introduces positive superhelical turns into DNA

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An enzymatic activity which converts closed circular DNA into a positively supercoiled form is present in cellular extracts of Sulfolobus, an acidothermophilic archaebacterium. This novel enzyme is a type II DNA topoisomerase and is active only at temperatures > 55 °C, in the presence of ATP and Mg²⁺.

THE topological state of DNA¹ affects its replication^{2,3}, recombination⁴⁻⁶, gene expression mediated by transcription^{7,8} and nucleosome assembly⁹⁻¹¹. DNA topoisomerases have been detected in a variety of organisms¹ and have been characterized and classified into two categories: type I topoisomerases transiently cut one of the two DNA strands to change the linking number in steps of one and type II topoisomerases act by means of a transient double-stranded break, which results in changing the linking number by two steps at a time. In eukaryotic cells, the type I enzyme, the so-called 'nicking-closing' enzyme, is predominant. This enzyme acts on both negatively and positively supercoiled DNAs, removing their superhelical turns. An ATP-requiring type II topoisomerase was first discovered by its particular ability to catenate and decatenate^{12,13} or to unknot the entangled DNA rings^{14,15}. This type II enzyme can also remove both negative and positive superhelical turns from DNA. In a typical bacterial extract, two types of topoisomerases are detected: ω -protein (type I) which relaxes negatively but not positively supercoiled DNA, and DNA gyrase (type II) which introduces negative superhelical turns into DNA¹⁶.

Archaeobacteria are believed to be a third type of organism¹⁷ which have various characteristics distinct from those of eukaryotes or prokaryotes.

We surveyed topoisomerase activities in extracts of *Sulfolobus*, an acidothermophilic archaebacterium, and found two remarkable features. First, in contrast to other organisms, ATP-requiring type II topoisomerase activities prevailed and were easily fractionated into three different enzymatic preparations, while type I topoisomerase activity was negligibly small. Second, one of the type II topoisomerases was able to introduce positive superhelical turns into DNA. We have tentatively named this new enzyme reverse gyrase and discuss possible implications of its biological function.

Identification of topoisomerases

Topoisomerase activities were surveyed in the following four conditions: (1) Relaxation of negatively supercoiled DNA was measured in the presence of EDTA, to detect eukaryotic type I nicking-closing activity. (2) The same conditions as for condition (1) but Mg²⁺ was added instead of EDTA, to detect ω -like activity (type I). (3) The same conditions as in condition (2) except that 1 mM ATP was included (type II). (4) Supercoiling of relaxed DNA was assayed in the presence of ATP and Mg²⁺, to detect a gyrase-like activity (type II).

Fig. 1 Fractionation of topoisomerase activities. Lane 1, the activity in the crude extract; all the other lanes show the activity in each fraction of the column.

Methods: The *Sulfolobus acidocaldarius* strain 7 (FERM P-7137, Fermentation Research Institute, Japan) used in this study was similar but not identical to *S. acidocaldarius* strain DSM 639 (ref. 32). The antiserum raised against pure RNA polymerase of the strain DSM 639 (provided by Dr W. Zillig) cross-reacted with that of our strain 7, although the molecular weights of each subunit examined by SDS-polyacrylamide gel electrophoresis were not identical

between the two strains (unpublished results). Cells were grown at 75 °C for 24 h with aeration in medium containing 0.1% yeast extract, 0.1% polypeptone, 0.1% casamino acids, 0.1% glucose, 0.2 g l⁻¹ NaCl, 1.3 g l⁻¹ (NH₄)₂SO₄, 0.3 g l⁻¹ KH₂PO₄, 0.25 g l⁻¹ MgSO₄, 0.07 g l⁻¹ CaCl₂ and 2.5–5 ml 1M H₂SO₄ to adjust the pH to 3.0. About 1.7 g (wet weight) of cells obtained from 2 l of culture were suspended in 50 mM Tris-HCl pH 7.5, 10% sucrose, 1 mM EDTA-Na₃ and 10 mM β -mercaptoethanol and disrupted by three cycles of freeze-thawing. Two successive centrifugations were done, first at 12,000 r.p.m. for 30 min to remove cell debris, then at 40,000 r.p.m. for 2 h to obtain a clear supernatant (crude extract). The crude extract was adsorbed to a DEAE-Sephacel column (1.2 × 6 cm) in the same buffer as above except that 10% glycerol was used instead of 10% sucrose, then fractionated with a linear gradient from 0.025 M to 0.5 M NaCl. Topoisomerase activity in each fraction was measured in a standard assay mixture (35 mM Tris-HCl pH 7.5, 1.8 mM spermidine-(HCl)₃, 0.14 mM EDTA-Na₃, 5 mM dithiothreitol, 24 mM KCl, 10 mM MgCl₂, 1 mM ATP, 90 μ g ml⁻¹ tRNA and 8.7 μ g ml⁻¹ of negatively supercoiled pBR322 DNA as a substrate) at 65 °C for 1 h. The reaction was stopped by adding 1% SDS, 10 mM EDTA-Na₃ and 5% glycerol, and the relaxation in superhelicity of pBR322 DNA was monitored by 1% agarose gel electrophoresis at room temperature in 89 mM Tris-base, 90 mM boric acid, 2.5 mM EDTA-Na₃. A ladder of DNA bands, each representing one topoisomer, was visualized after gels were stained in 0.5 μ g ml⁻¹ EtBr. The three peak fractions of relaxing activities shown here were pooled separately and adsorbed to a phosphocellulose column (0.6 × 5.5 cm) equilibrated with 20 mM K-phosphate pH 7.0, 10 mM β -mercaptoethanol, 0.1 mM EDTA-Na₃ and 10% glycerol. A linear gradient of NaCl (0–1.0 M) was applied to recover the topoisomerase activities. The active fractions Fr.2, Fr.3, Fr.4 and Fr.5 (described in Table 1) were dialysed against 50 mM Tris-HCl pH 7.5, 0.1 mM EDTA-Na₃, 10 mM β -mercaptoethanol and 50% glycerol. All steps were carried out at room temperature. Each fraction was stored at -20 °C and remained active for 2 yr.

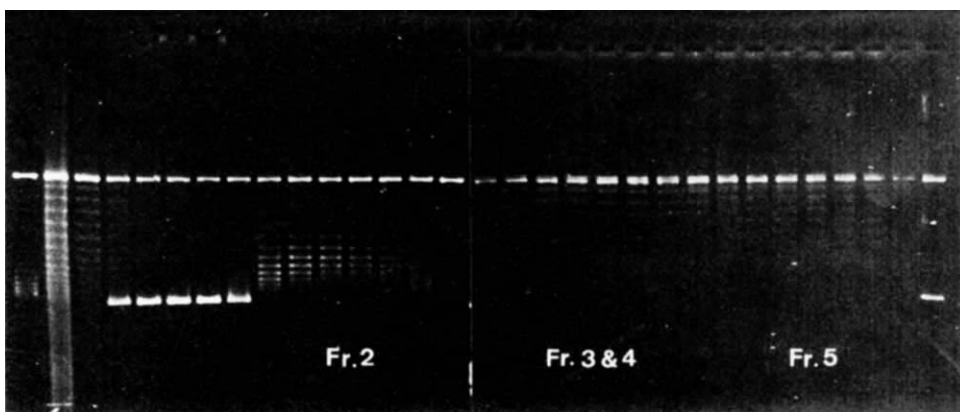


Table 1 Properties of the topoisomerases found in *Sulfolobus*

	DEAE-Sephacel (NaCl, M)	Phosphocellulose (NaCl, M)	MW $\times 10^3$	Requirement	Relaxation of negative super-coil	Relaxation of positive super-coil	Unknotting	Catenation	Supercoiling	Linking number change
Fr.2	0.1–0.15	0.4–0.5	200	Mg, ATP	+++	+++	+	+++	++	2
Fr.3	0.15–0.3	0	330	Mg	++	–	–	–	–	1
Fr.4	0.15–0.3	0.6–0.7	500	Mg, ATP	+++	–	+	+	+++	2
Fr.5	0.3–0.5	0	460	Mg, ATP	+++	+++	+++	–	–	2

The molecular weight (MW) of each topoisomerase activity was estimated by sedimentation in a 20–40% glycerol gradient at 45,000 r.p.m. for 20 h with the following internal markers: β -galactosidase, bovine serum albumin, haemoglobin and cytochrome *c*. The relaxation of positively supercoiled DNA was tested by adding excess EtBr to the standard assay mixture (see Fig. 1 legend). EtBr was extracted with *n*-butanol before agarose gel electrophoresis. The product of Fr.4 enzyme was also used as a positively supercoiled substrate. Unknotting activity¹⁴ was detected in the standard assay condition at 65 °C using knotted DNA extracted from P4 defective (tail-less) particles. A DNA band appeared at the position corresponding to the size of the P4 relaxed circle in the presence of ATP, whereas without enzyme or ATP no discrete bands were obtained. When incubation was at 75 °C, knotted DNA was pretreated with T4 DNA ligase to seal nicks at the cohesive ends¹⁵, otherwise a non-enzymatic reaction occurred. Catenating activity^{12,13} was judged from the DNA remaining at the top of the gel (see Fig. 3c, lane 4, for example), which was visualized as large aggregates under the electron microscope. Decatenating activity was measured by using these aggregates as a substrate.

As *Sulfolobus* grows at ~75 °C and not at temperatures <60 °C, incubation was at 65–75 °C for 1 h, after which the reaction was stopped by adding EDTA and SDS at room temperature, and the change in the linking number of closed circular DNA was monitored by agarose gel electrophoresis. No enzymatic activity in the crude extract was evident in conditions (1), (2) or (4), whereas a small amount of activity was detected in condition (3).

The crude extract was fractionated by DEAE-Sephacel chromatography. Figure 1 shows that activities in condition (3) above which relaxed negatively supercoiled DNA were detected in three separated fractions (Fr.). Each peak fraction was pooled and applied to a phosphocellulose column for further purification. The first peak and the third peak of the DEAE-Sephacel column exhibited single activities (Fr.2 and Fr.5), both requiring ATP. The second peak was divided into two fractions, one (Fr.3) active in the absence of ATP and the other (Fr.4) requiring ATP. The relaxation activity of each fraction sedimented at a unique position on glycerol gradient centrifugation, from which the molecular weight of each enzyme was estimated. Other activities such as supercoiling, unknotting of knotted P4 DNA^{14,15} and catenation^{12,13,15}, were assayed for each enzyme preparation.

The activity in Fr.4 is the main subject of this paper. The characteristics of the other topoisomerases are briefly summarized in Table 1 and will be reported in detail elsewhere.

Staggered ladders of DNA topoisomers

The enzymatic activity in partially purified Fr.4 was absolutely dependent on the addition of Mg^{2+} and ATP (Fig. 2a), and was demonstrably a type II topoisomerase¹⁶. The Fr.4 enzyme relaxed a DNA of unique negative superhelicity by two linking number changes at a time (Fig. 2b, lane 3). When relaxed DNA was used as a substrate, Fr.4 was able to introduce superhelical turns into DNA (Fig. 2c), showing ladders (a set of DNA topoisomers with different superhelicities) migrating more rapidly on agarose gel electrophoresis¹⁷. However, this gyrase-like activity was not evident at all with a DNA substrate of a defined negative superhelical density. Figure 2b, lane 3, shows that the Fr.4 enzyme could not negatively supercoil this substrate further but only relax it. By contrast, a more negatively supercoiled band was formed by the Fr.2 enzyme (Fig. 2b, lane 4), which served as a control for the gyrase activity. Furthermore, the position of each band of the ladders formed by the Fr.4 enzyme was slightly different and staggered compared with the

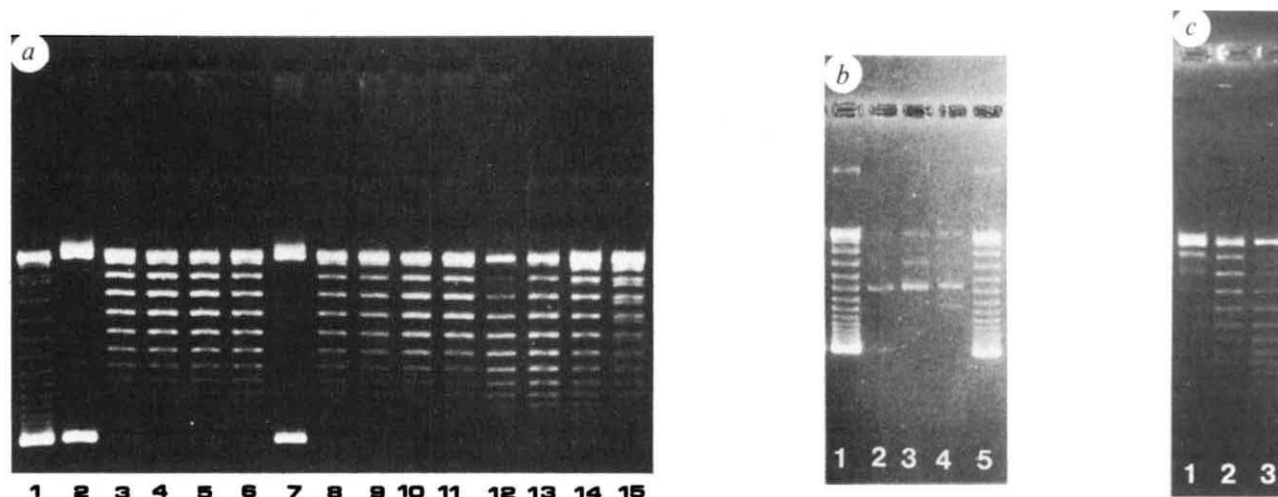


Fig. 2 Topoisomerase activity in Fr.4. *a*, Determination of the concentrations of Mg^{2+} , ATP and KCl optimal for Fr.4 activity. Each component was omitted from the standard assay mixture and then various amounts were added as indicated. Lane 1, pBR322 (negatively supercoiled topoisomers). Lanes 2–6, effect of Mg^{2+} concentration (0, 5, 10, 15 and 20 mM added in lanes 2–6, respectively). Lanes 7–11, effect of ATP concentration (0, 0.1, 0.2, 1 and 2 mM, respectively). Lanes 12–15, effect of KCl concentration (0, 50, 100 and 150 mM, respectively). *b*, Change of the linking number. A DNA band corresponding to a unique negative superhelicity was extracted from the ladder in agarose gel, and incubated with Fr.4 in conditions chosen to produce as minimal a change in linking number as possible. pBR322 DNA with a unique superhelical density (lane 2) was incubated with Fr.4 (lane 3) or Fr.2 (lane 4). The Fr.2 enzyme serves as a control demonstrating the linking number change by +2 and –2. Lanes 1 and 5 represent markers of negatively supercoiled topoisomers of pBR322. *c*, Supercoiling activity. Relaxed pBR322 DNA (lane 1) was used as a substrate in the standard condition and was incubated with Fr.4 at 65 °C (lane 2) or 75 °C (lane 3).

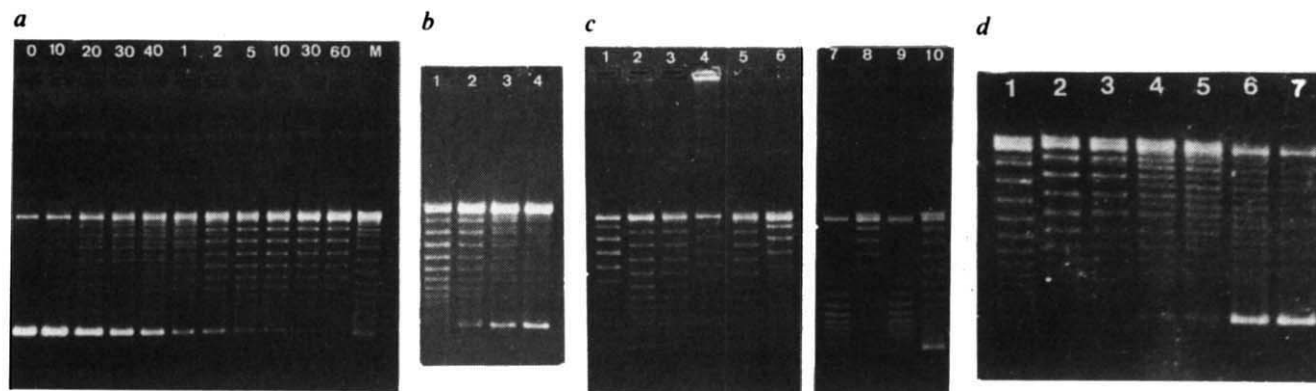
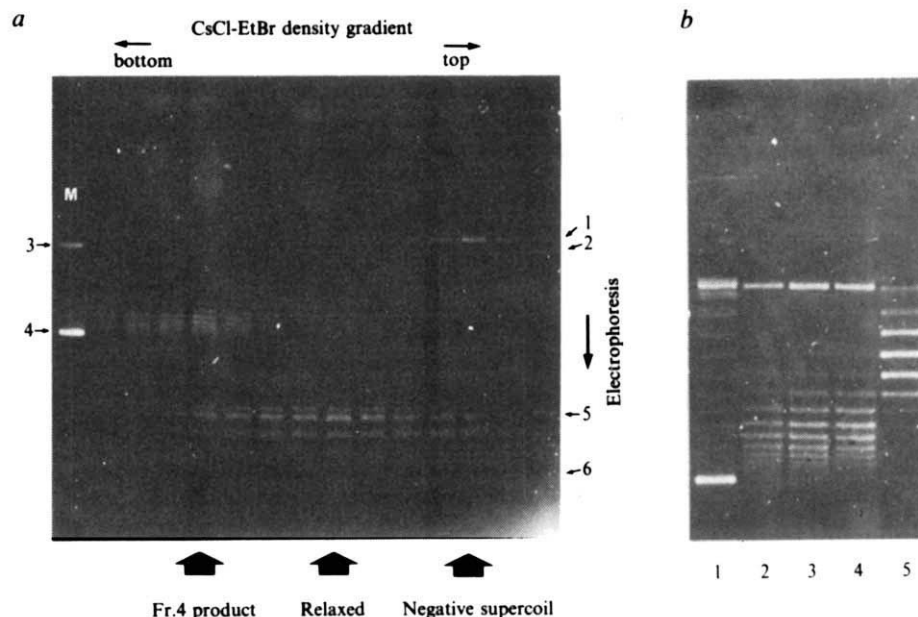


Fig. 3 Staggered ladders formed by Fr.4 enzyme. *a*, The time course of Fr.4 activity was monitored in standard assay conditions using 0.3 μ g of Fr.4 protein and 0.26 μ g of negatively supercoiled pBR322 DNA as a substrate. The reaction was stopped at the time indicated at the top of the column: 0, 10, 20, 30, 40 s, then 1, 2, 5, 10, 30, 60 min. The lane labelled M shows negatively supercoiled topoisomers of pBR322 prepared by yeast nicking-closing enzyme. *b*, Fr.4 activity was examined by changing the enzyme concentration. The amount of protein in each reaction mixture was (lanes 1–4, respectively): 0.15 μ g, 0.03 μ g, 0.015 μ g and 0.003 μ g. *c*, Interconversion of staggered ladders by other topoisomerases. Lane 1, a set of negatively supercoiled pBR322 topoisomers prepared with Fr.2 (standard ladder). Lane 2, Fr.4 was added to the sample prepared as in lane 1 and incubated further. The ladders were staggered. Lane 3, the reaction product of Fr.4 (staggered ladders). Lane 4, Fr.2 was added to lane 3 and incubated further. The ladder converted to the standard one and the catenated DNA also. Lane 5, same as lane 3. Lane 6, Fr.5 was added to lane 5 and incubated further. Lane 7, same as lane 3. Lane 8, yeast nicking-closing enzyme was added to lane 7 for further incubation at 30 °C. Lane 9, *E. coli* ω -protein was added to lane 7 for further incubation at 37 °C. Lane 10, a set of negatively supercoiled pBR322 topoisomers prepared by yeast nicking-closing enzyme (standard ladder). *d*, Catalytic nature of the enzyme. pBR322 DNA (0.26 μ g) was included in each reaction mixture, while the amount of protein in the purified enzyme fraction was (lanes 2 to 7, respectively) 0.012 μ g, 0.006 μ g, 0.003 μ g, 0.0015 μ g, 0.0006 μ g and 0.0003 μ g. Lane 1 serves as a marker of negatively supercoiled topoisomers of pBR322 prepared by yeast nicking-closing enzyme (at 30 °C for 10 min). Note that a nicked circle, a relaxed circle, and supercoils with one positive turn and with one negative turn overlap with the two topmost bands of the ladders. The marker (lane 1) is rich in negatively supercoiled (–1 turn) and relaxed circles, while many nicked circles are found in the sample incubated at 75 °C (lanes 2–7). This explains a slight difference in the position of the top band in lane 1.

Methods: *d*, Fr.4 was further purified on a hydroxylapatite column equilibrated with 0.1 M phosphate buffer (pH 7.0) containing 0.25 M NaCl. The topoisomerase activity was eluted by a linear gradient of phosphate (0.1–1.0 M). An active fraction recovered at 0.35 M phosphate was used for this experiment. The protein concentration was measured using Bio-Rad protein assay reagents. As the molecular weight of the enzyme was estimated to be ~500,000 (close to the β -galactosidase marker in glycerol gradient centrifugation), the molar ratio of protein to pBR322 DNA was calculated to be (lanes 2–7, respectively): 0.24, 0.12, 0.06, 0.03, 0.012 and 0.006.

Fig. 4 CsCl–EtBr density gradient centrifugation. *a*, The first lane (indicated by M) is a size marker for pBR322 DNA. All the other lanes correspond to fractions from the CsCl–EtBr density gradient. Only an appropriate part of the gel is shown, where covalently closed circular DNA is present. The numbers indicate the positions of the following DNAs: (1) dimeric pBR322 (negatively supercoiled), (2) pBR322 (nicked circle), (3) pBR322 (relaxed circle), (4) pBR322 (negatively supercoiled), (5) pAO3 (relaxed or nicked circle), (6) pAO3 (negatively supercoiled). *b*, The pattern of the pBR322 DNA in the peak fraction compared with that of standard samples of negatively or positively supercoiled DNA. Lane 1, negatively supercoiled topoisomers of pBR322. Lanes 2–4, peak fractions of positively supercoiled pBR322 shown in *a*. Lane 5, positively supercoiled topoisomers of pBR322.

Methods: *a*, Negatively supercoiled pBR322 (4.3 kilobases, kb) treated with Fr.4 enzyme, was mixed with negatively supercoiled pAO3 (1.8 kb) DNA treated with Fr.5 enzyme (a marker for relaxed DNA at 75 °C) and a negatively supercoiled dimer of pBR322 (8.6 kb) (a marker for negatively supercoiled DNA at 37 °C). The solution was adjusted to 3.7 ml in 10 mM Tris-HCl pH 7.8, 10 mM EDTA–Na₃. After adding 3.8 g of solid CsCl and 0.3 ml of 5 mg ml^{–1} EtBr, the mixture was centrifuged in the RTV65 vertical rotor of the Hitachi ultracentrifuge (35,000 r.p.m. at 20 °C for 48 h). The gradient was fractionated from the bottom and DNA was extracted twice with *n*-butanol. After dialysis against 10 mM Tris-HCl pH 8, 1 mM EDTA–Na₃, each fraction was analysed by 1.0% agarose gel electrophoresis. *b*, Positively supercoiled topoisomers were prepared by incubating negatively supercoiled pBR322 DNA with yeast nicking-closing enzyme at 4 °C for 24 h. The resultant molecules were positively supercoiled when assayed at 24 °C (ref. 19).



negatively supercoiled topoisomers prepared by yeast nicking-closing enzyme (Fig. 2a, compare lane 1 with the rest).

In favourable conditions of gel electrophoresis, the staggered bands formed by Fr.4 enzyme migrated just between the standard array of negatively supercoiled topoisomers, prepared either by yeast nicking-closing enzyme or Fr.2 or Fr.5 enzyme (compare

Fig. 3c, for example). The time course of the reaction with Fr.4 enzyme (see Fig. 3a) demonstrated that in a short incubation time, two sets of the ladders, regular and staggered, appeared and as time progressed, the former bands disappeared and the latter ones intensified. A similar pattern was observed with decreasing amounts of enzyme, where doublet ladders were evident (see Fig. 3b). These results suggested that staggered ladders were formed via standard ladders when the reaction proceeded further. The pattern of the staggered ladder was not affected by further deproteinization with phenol and chloroform-isoamyl alcohol. It converted to a normal (negatively supercoiled) ladder after treatment with Fr.2 enzyme, which contained DNA gyrase and DNA catenase activity (Fig. 3c, lanes 3, 4), or with Fr.5 enzyme, a type II topoisomerase which could relax both negative and positive supercoils (Fig. 3c, lanes 5, 6). Therefore, this staggered ladder must be attributed to the DNA topoisomers. The reaction product of Fr.4 enzyme was next treated with either yeast nicking-closing enzyme or *Escherichia coli* ω -protein. The former catalysed the relaxation of staggered ladders but the latter could not (Fig. 3c, lanes 7–9). As distinct from the yeast enzyme, ω -protein does not relax positively supercoiled DNA. This result suggests that the staggered ladders comprise positively supercoiled DNA topoisomers.

Positively supercoiled topoisomers

To distinguish between the various possible conformations of DNA, the reaction product of Fr.4 was centrifuged in a CsCl equilibrium density gradient in the presence of ethidium bromide (EtBr) then each fraction was analysed by agarose gel electrophoresis. Figure 4a shows that pBR322 DNA incubated with Fr.4 enzyme formed a band of much heavier density than the relaxed form of pAO3 DNA¹⁸ (prepared by Fr.5 enzyme) which was used as an internal marker, indicating that the former represented positively supercoiled DNA. The ladders at the peak position of pBR322 (Fig. 4b, lanes 2–4) coincided with a standard sample of positively supercoiled topoisomers (lane 5)¹⁹ but not negatively supercoiled ones (lane 1).

Two-dimensional agarose gel electrophoresis²⁰ provides another means of distinguishing between positive and negative superhelical DNA topoisomers. After electrophoresis in the first dimension, gels were soaked in a mild concentration of EtBr and electrophoresed in the orthogonal direction. In the second dimension, positively supercoiled DNA had a higher mobility due to the increase in the superhelical density by EtBr, while the migration of negatively supercoiled DNA was retarded because EtBr relaxed it. Figure 5a shows clear separation of a control mixture of negatively and positively supercoiled DNA topoisomers in the second dimension, forming a symmetrical arch pattern. The reaction products of Fr.4 enzyme (Fig. 5b) migrated more rapidly in the second dimension, indicating that they were in fact positively supercoiled DNA. In Fig. 5c, a mixture of pAO3 incubated with Fr.4 enzyme and pBR322 treated with Fr.5 enzyme (a control for relaxed DNA at 75 °C) was developed by two-dimensional agarose gel electrophoresis. The pattern of pAO3 clearly showed the formation of positively supercoiled DNA topoisomers.

Superhelicity at high temperature

It should be noted that our reaction temperature was 75 °C and that the products were analysed at 24 °C. The superhelicity of DNA is known to be affected by temperature¹⁹. Thus, in the conditions of our analysis, the DNA would be less positively supercoiled, or more negatively supercoiled, than in the reaction mixture. The difference in superhelicity caused by temperature was roughly three superhelical turns per 20 °C in our system using pBR322 (data not shown). Note also that the fully relaxed pBR322 DNA formed by the topoisomerase in Fr.5 at 75 °C (see Table 1) appeared to be negatively supercoiled by five turns in our assay conditions. DNA must be more positively supercoiled by several turns in our reaction mixture at 75 °C than was actually observed at the ambient temperature.

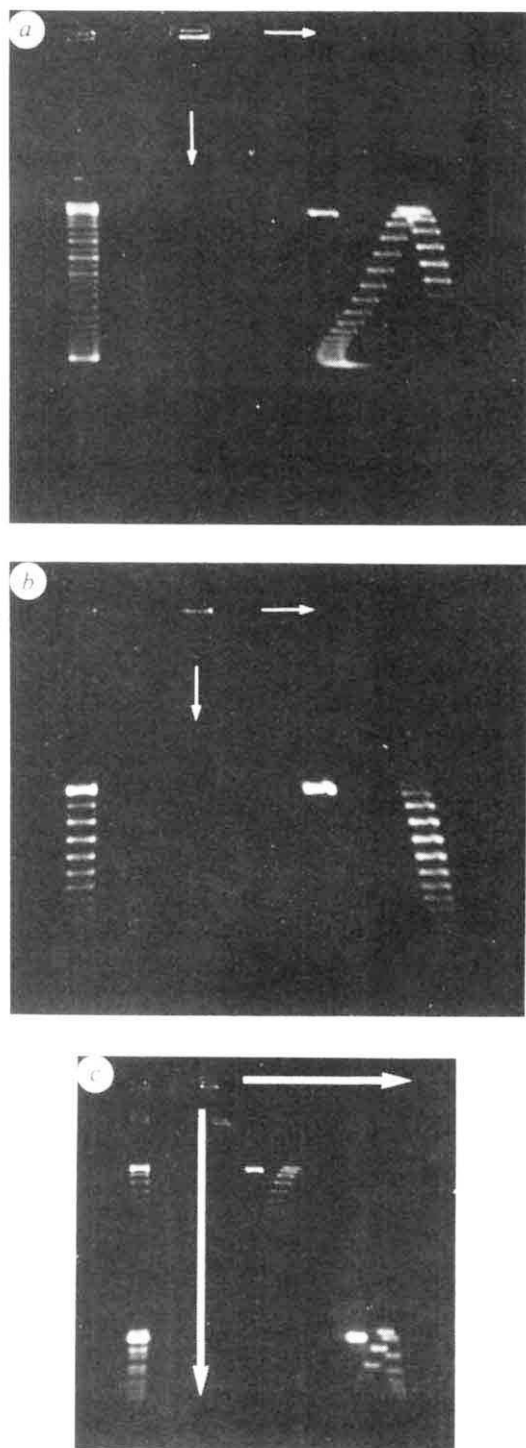


Fig. 5 Two-dimensional agarose gel electrophoresis. *a*, A mixture of negatively supercoiled pBR322 and positively supercoiled pBR322 prepared as described for Fig. 4b was electrophoresed in the first dimension in the standard conditions. The gel was soaked in $0.02 \mu\text{g ml}^{-1}$ EtBr solution for 90 min and re-electrophoresed in the orthogonal direction in the same buffer containing $0.02 \mu\text{g ml}^{-1}$ EtBr. *b*, pBR322 DNA treated with Fr.4 enzyme was electrophoresed in the same way as for *a*. *c*, A mixture of pBR322 treated with Fr.5 enzyme and pAO3 treated with Fr.4 enzyme was analysed as described above.

Reverse gyrase—a novel enzyme?

The reverse gyrase studied here might consist of two components: a putative DNA-binding protein which introduces positive superhelical constraints, consequently creating negatively supercoiled domains in the unoccupied parts of closed circular DNA, and a topoisomerase which could relax these regions. The resulting DNA would then be positively supercoiled on removal of the binding proteins. This type of mechanism was excluded by the following two experiments.

First, the minimal protein concentration required for reverse gyrase activity (for this purpose, Fr.4 was further purified by hydroxylapatite chromatography) was estimated to be less than one-tenth the molar ratio of the substrate DNA (Fig. 3d). This amount of protein in the reaction mixture is insufficient to constitute a nucleosome-like complex, as DNA-binding proteins would be needed in a molar excess over the DNA. A preliminary analysis of protein components in this fraction by SDS-polyacrylamide gel electrophoresis revealed four major bands in equimolar amounts (molecular weights 124,000, 101,000, 45,000 and 42,000). Second, the linking number of DNA in the hypothetical protein-DNA complex should not be changed by adding exogenous topoisomerases as the DNA should be relaxed before deproteinization, while free positively supercoiled DNA should be a good substrate for the second topoisomerase. This notion was tested by further incubation of the reaction product of Fr.4 with Fr.5 enzyme at 75 °C or with yeast nicking-closing enzyme at 30 °C. Figure 3c, lanes 5–8 shows that the final products were relaxed DNAs, indicating that the positively supercoiled DNA was not complexed in the reaction mixture. Therefore, the Fr.4 activity was not due to a combination of DNA-binding proteins and a topoisomerase, but Fr.4 must contain the unique enzymatic activity of a reverse gyrase which produces DNA with positive superhelical tension.

In living organisms, no positively supercoiled DNA has ever been reported, nor has an enzyme been found to produce such a topological constraint. It is possible that positive supercoils are relaxed as soon as they are formed, by the potent type I topoisomerases generally found in eukaryotic cells. In *Sulfolobus*, type I topoisomerase activity was low compared with the three other topoisomerases (see Table 1), which may have been essential for our discovery of the novel reverse gyrase activity. Positively supercoiled DNA can be prepared *in vitro* by: (1) using intercalating drugs such as EtBr; (2) raising the temperature of a solution containing relaxed circular DNA prepared at a lower temperature; (3) binding large amounts of DNA gyrase to nicked DNA, then sealing it and removing the proteins²¹; (4) using stoichiometric amounts of DNA topoisomerase II²²; or (5) using netropsin and a DNA topoisomerase²³. Here, we have shown that reverse gyrase can form positively supercoiled DNA by changing the linking number of closed circular DNA. The biological activity of such a DNA molecule is of great interest, as well as its physicochemical properties.

Compared with the extent of the positive superhelicity produced by intercalating pBR322 DNA with EtBr (more than 20

turns), the superhelicity produced by reverse gyrase appeared incomplete. We observed positively supercoiled DNA with eight turns in our gel analysis at 25 °C (see Fig. 3c, lane 7 and Fig. 4b, lane 3, for example), thus we estimate 13 positive superhelical turns at best in our reaction at 75 °C. There are two possible explanations for this: first, there is an upper limit to increasing the linking number of DNA because of some physical stress in the DNA helix itself. The positively supercoiled circles formed by netropsin binding, described above, represent the same situation²⁴. Second, as the DNA becomes more positively supercoiled, so its ability to react with the enzyme might decrease.

Why is reverse gyrase needed?

Negatively supercoiled DNA is believed to be a favourable substrate for replication, transcription and recombination. Conversely, positively supercoiled DNA might be unfavourable for these functions. So what is the role of a topoisomerase which introduces positive superhelical strains into DNA? One trivial explanation is that because *Sulfolobus* grows at 75 °C, reverse gyrase is required to prevent the genomic DNA from denaturation by tightening the helical pitch of DNA (as the G+C content of *Sulfolobus* is only 40%). If this is the case, similar activities should exist in other thermophilic organisms, and their plasmids, if any²⁵; no such examples have been reported.

We suggest, on the other hand, that reverse gyrase is distributed widely in many organisms to counteract the effects of other topoisomerases. It may actively alter the superhelicity of DNA so as to disassemble the stable complexes of proteins and negatively supercoiled DNA. For example, chromatin DNA is held in a stable complex with histones but it is believed that DNA must be freed from histones for replication, recombination, transcription, etc. To release the histones from nucleosomes it may be necessary to reverse the assembly process²⁶, in which positive superhelical turns have been removed by topoisomerases. Instead, the reverse gyrase could introduce positive superhelical turns into the internucleosomal regions and the resulting superhelical strain could detach the histones from the DNA.

A similar situation may prevail when cruciform structures or segments of Z-DNA²⁷ that are formed in the negatively supercoiled DNA^{28,29} need to be reorganized to regular B-form DNA. Relaxing the negatively supercoiled DNA may not provide sufficient driving force to cause structural changes (probably because specific proteins must be associated) and here reverse gyrase may be needed. The transition of such structures in chromosomes may be important as initiation signals for DNA replication, recombination or gene expression^{30,31}.

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Use of light for footprinting DNA *in vivo*

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A new 'photofootprinting' technique, which uses light to detect protein-DNA contacts as well as changes in the structure of DNA at the base pair level, has been developed and used to detect contacts between lac repressor and the lac operator in Escherichia coli cells.

ALTHOUGH it is well established that interactions between DNA and protein regulate gene expression, there are few techniques capable of directly probing these interactions *in vivo*. Given the complexity of the intracellular environment and our inability to reconstruct it, accurate molecular descriptions of gene regulation are critically dependent on the development of such techniques. Currently, experiments using DNase I 'footprinting'¹, chemical modification^{2,3}, bromouracil cross-linking⁴ and drug cleavage^{5,6} can probe, with high resolution, protein-DNA interactions *in vitro*. In each technique a protein is bound to a uniquely end-labelled DNA fragment. The complex is then subjected to an enzymatic, chemical or photochemical procedure which either breaks the DNA backbone directly or modifies the DNA in such a way that its backbone can be subsequently broken by alkali⁷. After removal of protein, the labelled DNA is denatured and electrophoresed in a polyacrylamide sequencing gel. An autoradiograph of this gel shows a pattern of end-labelled fragments which correspond to breaks in the DNA backbone induced by the modification or cleavage procedure. As cleavage rates may be altered by protein binding, corresponding changes in the strand breakage pattern may be used to identify protein contacts.

Although protein binding sites can be identified and characterized *in vitro* by these techniques, the chemical or enzymatic procedures used to induce strand cleavage are often too harsh and too slow to be used on living cells without perturbing native protein-DNA interactions. We report here the development of a new technique, termed 'photofootprinting', which uses light to probe protein-DNA interactions *in vitro* and *in vivo*. The technique, in its simplest form, is based on the observation that a small but significant distortion of the double helix is necessary to form a UV photoproduct⁸⁻¹¹. If protein contacts affect the geometrical changes necessary for photoproduct formation, exposure of protein-free and protein-bound DNA to light followed by breakage of the DNA backbone only at the sites of photodamage will yield two different sets of DNA fragments. When analysed on a sequencing gel, differences in the strand-breakage pattern of these two DNAs will occur at those bases in contact with protein. More elaborate procedures based on photochemical reactions between DNA and small probes such as photosensitizers will be described below.

To detect protein-DNA contacts using light, we have developed a chemical procedure which exploits a common feature of photoreacted bases absent in unmodified bases so as to break the DNA backbone only at the sites of photodamage. In each of the known major photoproducts in DNA, one or more of the 5,6-double bonds of a pyrimidine becomes saturated on photoreaction^{8,12}. As carbonyl groups conjugated to a double bond are poorly reduced by sodium borohydride¹³, the carbonyl group at position 4 of photochemically modified thymines can be selectively reduced in the presence of other bases¹⁴. Furthermore, Kunieda and Witkop¹⁵ and Miller and Cerutti^{14,16}, have shown that reduction of thymines saturated at the 5,6-position with excess NaBH₄ opens the thymine ring. As conjugation of the lone pair electrons of the glycosidic nitrogen at position 1 is greatly reduced by saturation of the 5,6-double bond and ring opening, the glycosidic bond of photochemically modified and reduced thymine should be much more labile to acid hydrolysis

than unmodified thymine. Once the glycosidic bond is hydrolysed by acid, the phosphate backbone can be broken at the sugar ring by subsequent treatment with alkali⁷. In practice, base hydrolysis and subsequent breakage of the backbone can be carried out in one step by briefly heating in acidic aniline.

Although the 4 position of cytosine is not reduced by NaBH₄, saturation of the 5,6-double bond is known greatly to enhance spontaneous deamination of the 4 position, converting it to a carbonyl group¹⁷. Thus, like thymine, photochemically modified cytosine should be selectively reduced and its ring opened by NaBH₄, labilizing its glycosidic bond to acid hydrolysis and subsequent strand breakage.

Photofootprinting the *lac* repressor *in vitro*

To determine whether protein contacts can be detected with UV light alone, a 95-base pair (bp) fragment of DNA containing the *lac* operator¹⁸ was exposed for 20 and 80 s to a 150-W xenon lamp in the presence and absence of *lac* repressor. After purification and after the DNA was end-labelled with ³²P, the photoproducts were labilized by the chemical degradation procedure and the resulting strand breakage pattern was analysed on a sequencing gel. In the absence of *lac* repressor, brief irradiation of the *lac* operator induced the formation of many photochemically modified bases which could be detected by the chemical degradation procedure (Fig. 1). Photoproducts were detected not only at pyrimidines but, unexpectedly, also at purines. Although photoreactions between adjacent pyrimidines are well documented⁸, photoreactions between pyrimidines and purines are not¹², and further characterization is necessary to establish the identity of these new photoproducts.

When these experiments are repeated in the presence of *lac* repressor, repressor binding inhibited, and in some cases enhanced the ability of base pairs to undergo photochemical modification (Fig. 1). The location of the major repressor contacts is in excellent agreement with the repressor binding site detected by methylation protection¹⁹, DNase I footprinting²⁰, bromouracil cross-linking⁴ and chemical synthesis methods²¹. Thus, brief exposure of the *lac* repressor-*lac* operator complex to UV light can be used to identify repressor contacts.

In contrast to other techniques^{19,20}, UV light also detects repressor contacts upstream and downstream from the known repressor binding site. Although these weak 'contacts' could result from repressor-induced distortions in adjacent protein-free DNA, we believe them to represent previously unreported or unidentified repressor contacts (see below).

Figure 2 examines in detail the chemistry used to detect the *lac* repressor-*lac* operator photofootprint. Several features of this analysis are noteworthy. First, detection of a photofootprint requires both NaBH₄ reduction and acidic aniline cleavage. If either step is excluded, no cleavage of the DNA is observed (compare methods *d* and *e* and *b* and *c*). Second, in the absence of photodamage, cleavage of DNA by the NaBH₄-acidic aniline technique is very weak and comparable with the same background cleavage resulting from piperidine treatment (compare the first lanes of methods *a*, *b* and *e*). Thus, the background cleavage of the NaBH₄-acidic aniline technique is as low as the background cleavage observed in the chemical sequencing method of Maxam and Gilbert⁷. Third, in these conditions UV light does not directly cleave DNA (method *a*).

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Fig. 1 *In vitro* photofootprints of the *lac* repressor bound to the *lac* operator. **a**, An autoradiogram of the *lac* repressor photofootprint. Lane 1, a no-light control showing the background resulting from the chemical labilization procedure. Lanes 2, 3, the effect of irradiating the *lac* operator for 20 and 80 s, respectively, in the absence of *lac* repressor. Lanes 4, 5, the effect of irradiating the *lac* operator for 20 and 80 s, respectively, in the presence of *lac* repressor. The level of photodamage in these experiments is very low: ~1 bp in 500 is damaged by light. Arrows denote bases whose photodamage is totally blocked by repressor binding. **b**, results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The upper half summarizes the results of the 20-s irradiation experiment and the lower half those of the 80-s irradiation experiment. The effect of light on the cleavage of DNA in the absence of *lac* repressor is expressed as the ratio $(+hv/-R')$, where $+hv$ is the cleavage of a base in the presence of light and $-R'$ is its cleavage in the absence of light. The effect of added *lac* repressor on light-induced cleavage of a base is expressed as the ratio $(+R'/-R')$ where $+R'$ is the light-induced cleavage of a base in the presence of *lac* repressor minus its cleavage in the absence of light and $-R'$ is the cleavage of the base in the absence of repressor minus its cleavage in the absence of light. When the value of $(+R'/-R')$ is $<1/20$, protein binding is considered to block photodamage totally and the corresponding position in the histogram is blackened. In brackets, that region of the *lac* operon protected by *lac* repressor from DNase I digestion is shown as a pair of lines²⁰. The upper line summarizes the protection results for the same DNA strand analysed in the photofootprinting experiments and the lower line summarizes the results for the opposite strand. That region of the *lac* operator protected by *lac* repressor from DMS is shown below the DNase I results¹⁹. Strong protection is denoted by a continuous line and weak protection by a dashed line.

Methods: Irradiation was conducted with a 150-W xenon lamp (PRA model ALH215) equipped with an elliptical mirror. The output of the lamp consists of a focused beam 3 mm in diameter which is passed through a 10-cm long water filter to remove IR radiation before impinging on the sample. Samples were irradiated in 200 μ l Micropets (Clay Adams) sealed at one end with a rubber septum. Although the Micropets absorb a small amount of UV radiation, similar results were obtained in UV-transparent cuvettes. In a typical experiment, 12 μ l of a solution consisting of 10 mM Tris (pH 7.4), 10 mM Mg(OAc)₂, 10 mM KCl, 0.1 mM EDTA, 0.1 mM 2-mercaptoethanol, 12% glycerol, 100 μ g ml⁻¹ gelatin, 15 ng of a 95-bp *lac* operator fragment¹⁸ and 0.1 μ g of pBR322 plasmid DNA was irradiated in the absence or presence of 1.4 μ g of *lac* repressor. 10 μ g of carrier tRNA was then added to each sample and repressor removed by phenol extraction. The *Eco*RI end of the *lac* operator fragment was labelled with 25 μ Ci of [α -³²P]dATP using the Klenow fragment of *E. coli* DNA polymerase I and unreacted ATP was removed by dialysis against 2 M NaCl, 1 mM Tris (pH 7), followed by dialysis against 1 mM Tris (pH 7). The solutions were transferred to a 1.5-ml Eppendorf tube and heated at 50 °C for 5 h in 1 mM Tris (pH 7), 5 mM NaCl to effect deamination of photochemically modified cytosines. After precipitation from ethanol, each sample was dissolved in 50 μ l of 5 mM KP_i (pH 8.3), 5 mM NaCl containing 50 mM thymidine and cooled to 0 °C. 50 μ l of the same KP_i/NaCl/thymidine solution containing 1 mg of freshly dissolved sodium borohydride was then added. The samples were briefly vortexed and allowed to stand unagitated and open to the air at 2 °C in the dark. Thymidine is present during the reduction step to react with any borane generated by the decomposition of NaBH₄ in water. Otherwise, borane, which preferentially reduces carbonyl groups conjugated to double bonds, would reduce those bases that are not damaged by light. The reactions were stopped after 15 h by adding 250 μ l of 0.7 M NaOAc-HOAc (pH 5.0) and allowed to stand at room temperature for 1 h with occasional vortexing. After precipitating the DNA from 750 μ l of ethanol the dried and rinsed pellets were redissolved in 170 μ l of 0.5 M NaOAc-HOAc (pH 5.0). Following incubation at room temperature for 1 h with occasional vortexing, 100 μ l of water and 750 μ l of ethanol were added to precipitate the DNA. The rinsed and dried DNA pellets were then dissolved in 2 μ l of water and 25 μ l of 1.0 M aniline-HOAc (pH 4.5) was added^{39,40}. After heating at 60 °C for 20 min in the dark, the samples were frozen at -78 °C and lyophilized under high vacuum. Freezing and lyophilization from 25 μ l of water was repeated three more times and the DNA twice from ethanol. Following rinsing and drying of the DNA pellet, the samples were dissolved in 98% deionized formamide, boiled for 3 min and electrophoresed on a 20% polyacrylamide sequencing gel at 1,500 V for 14 h.

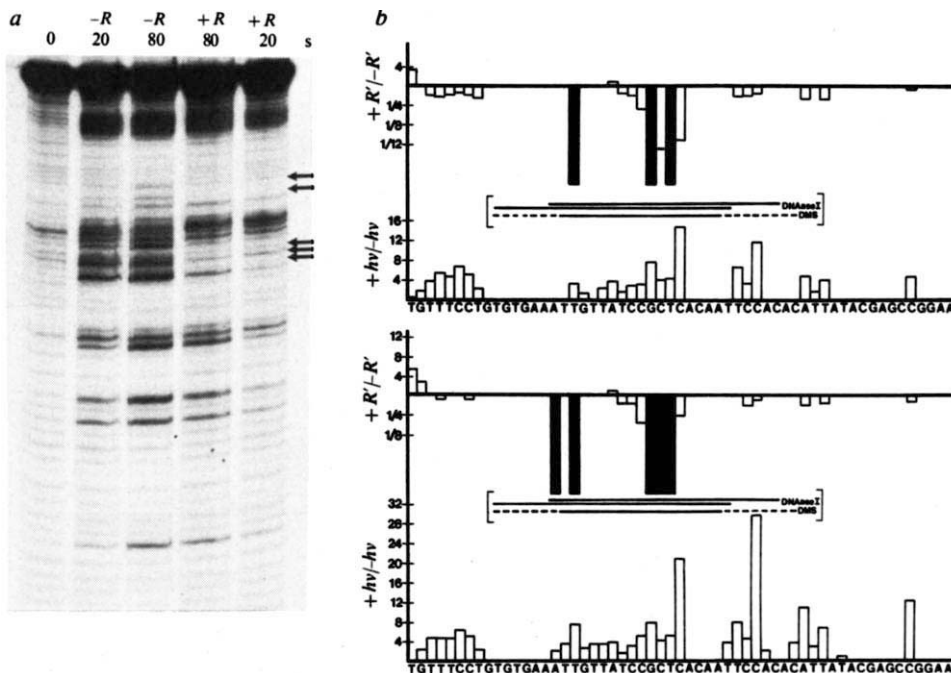
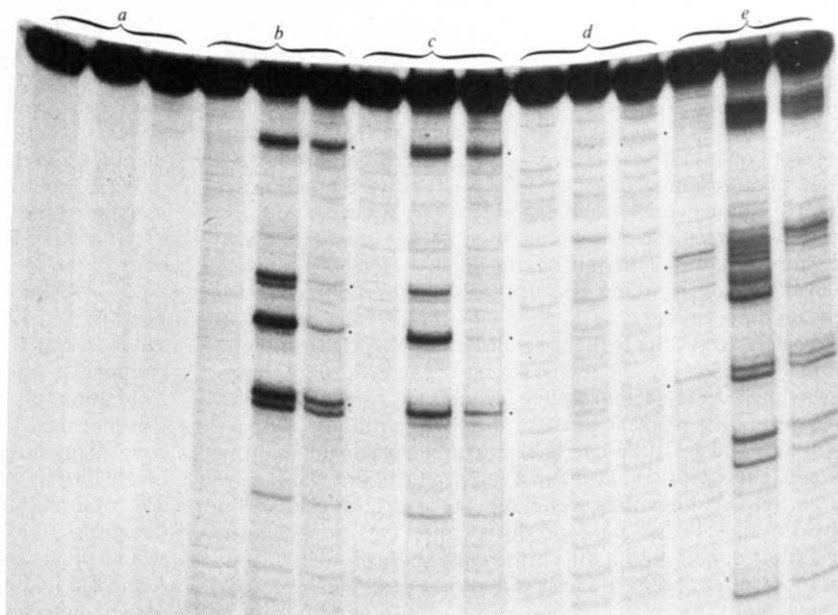


Fig. 2 *In vitro* photofootprints of the *lac* repressor bound to the *lac* operator detected by different chemical treatments (**a-e**). For each chemical treatment, three lanes are shown. The first lane of each set is a no-light control, the second lane shows the effect of irradiating the *lac* operator for 40 s in the absence of *lac* repressor and the third lane shows the effect of irradiating the *lac* operator for 40 s in the presence of *lac* repressor. **a**, No chemical treatment; **b**, cleavage is induced by heating in piperidine; **c**, cleavage is induced by successively deaminating, reducing with NaBH₄ and heating in piperidine; **d**, cleavage is induced by successively deaminating and heating in acidic aniline; **e**, cleavage is induced by successively deaminating, reducing with NaBH₄ and cleaving with acidic aniline.

Methods: DNA was cleaved with piperidine by dissolving the DNA in 100 μ l of freshly diluted 1 M piperidine and heating at 90 °C for 25 min⁷.

All other methods are identical to Fig. 1.



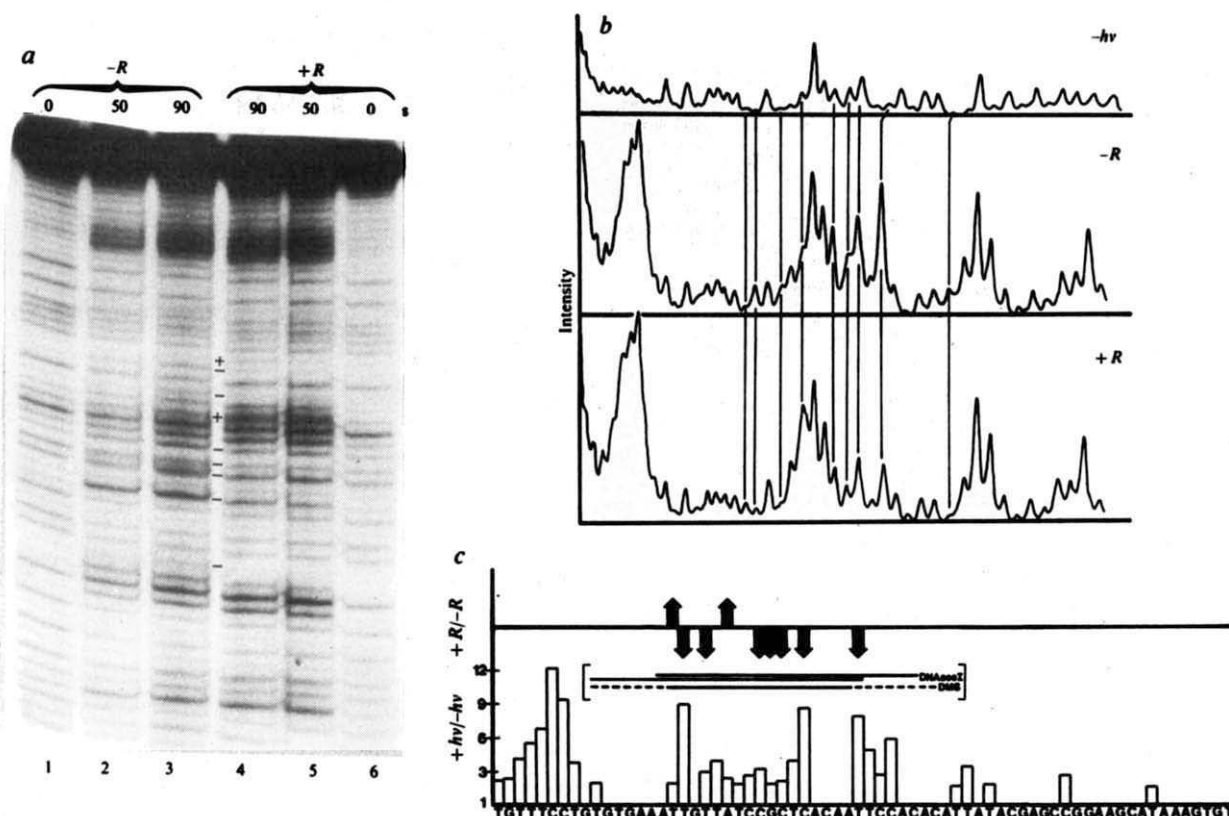


Fig. 3 Photofootprints of the *lac* repressor bound to the *lac* operator *in vivo*. **a**, An autoradiogram of the *lac* repressor photofootprint obtained *in vivo*. Lanes 1–3, the effect of irradiating *E. coli* cells containing the *lac* operator plasmid unbound by *lac* repressor for 0, 50 and 90 s. Lanes 4–6, the effect of irradiating *E. coli* cells containing the *lac* operator plasmid bound by repressor for 90, 50 and 0 s, respectively. **b**, Microdensitometer scans of the 90-s irradiation experiment. The upper portion is the no-light control (lane 6 of **a**; similar results are also observed in lane 1). The middle and lower portions show the effect of irradiating the *lac* operator plasmid in the absence and presence of bound repressor, respectively (lanes 3, 4). **c**, The microdensitometer scans quantified and displayed as a histogram. The effect of light on the cleavage of DNA in the absence of *lac* repressor is expressed as the ratio $(+hv/-hv)$ while the effect of repressor on light-induced cleavage of DNA is denoted either as an enhancement ($\uparrow$) or blockage ($\downarrow$) of cleavage.

Methods: Competent *E. coli* cells lacking a chromosomal copy of the *lac* structural gene (*E. coli* strain E7074 F⁺lacIProA⁺, B⁺Δ(*lac pro*) sup *E. thi* (kindly provided by Dr J. Beckwith) were transformed with either plasmid pJW231 or pJW280 (see text for explanation). 900 μl of *E. coli* cells bearing either plasmid were grown to saturation in LB, pelleted and resuspended in 450 μl of M9 buffer⁴¹. The cells were then placed on ice and photolysed in 150-μl aliquots in a quartz microcuvette. Following irradiation, each aliquot was immediately frozen and stored at -78 °C. The aliquots were combined, pelleted and plasmid DNA isolated by an alkaline-SDS miniprep⁴². After phenol extraction from 1% SDS, the DNA was ethanol precipitated, restricted with *Eco*RI and labelled with [α -³²P]dATP using the Klenow fragment of *E. coli* DNA polymerase I. Unreacted ATP was removed by dialysis against 2 M NaCl, 1 mM Tris (pH 7), followed by dialysis against 1 mM Tris (pH 7). The DNA was then restricted with *Bam*HI, ethanol precipitated and the 95-bp *lac* operator fragment purified by electrophoresis on a 6% polyacrylamide gel. After extraction from the gel and ethanol precipitation, the DNA was processed and analysed as described in Fig. 1 legend.

Although the NaBH₄-acidic aniline technique can be used to break the DNA backbone at the sites of photodamage, the efficiency with which the photoproducts are detected is unknown. To estimate this efficiency, a second cleavage technique has been used to labilize the *lac* repressor-*lac* operator photofootprint described in Fig. 1. As shown by others, when UV-irradiated DNA is heated in piperidine the DNA backbone is cleaved quantitatively at the so-called PydC photoproducts²³. These photoproducts occur at T-C, C-C and to a much lesser extent T-T dinucleotide residues and result from a cyclo-addition reaction between the 5,6-double bond of one pyrimidine and the carbonyl group of thymine or the imino tautomer of cytosine of an adjacent pyrimidine. Because these photoproducts contain a pyrimidine saturated at its 5,6-double bond, their formation can be detected by the NaBH₄-acidic aniline cleavage technique outlined here. As Fig. 2 shows, cleavage of the PydC photoproducts by the NaBH₄-acidic aniline technique is comparable with the quantitative cleavage of these sites by piperidine (compare methods *b* and *e*). Although these measurements do not indicate the efficiency with which other photoproducts are cleaved by the NaBH₄-acidic aniline technique, the structural similarities between the PydC photoproducts and the well known pyrimidine dimers, pyrimidine trimers and spore product suggest that these latter photoproducts⁸ will also be readily labilized by this technique.

Photofootprinting the *lac* repressor *in vivo*

Having demonstrated the feasibility of footprinting a protein *in vitro* with light, we next investigated the possibility of footprinting *in vivo* with light. Two derivatives of the plasmid pBR322 have been constructed for this purpose. The first, designated pJW231, contains a copy of the same 95-bp *lac* operator fragment used in the *in vitro* experiments²⁴. The second, designated pJW280, contains this *lac* operator plus an 1,100-bp fragment containing the *lac* repressor structural gene under control of an *I*^q promoter. When *Escherichia coli* cells lacking a chromosomal copy of the *lac* repressor structural gene are transformed separately with these plasmids, two cell types result. *E. coli* cells bearing the pJW231 plasmid contain *lac* operators unbound by *lac* repressor whereas *E. coli* cells bearing the pJW280 plasmid contain *lac* operators bound by *lac* repressor. Following brief irradiation of both cells, plasmid DNA is isolated free of chromosomal DNA. The 95-bp *lac* operator fragment is excised from each plasmid by restriction with *Eco*RI and *Bam*HI, end-labelled with ³²P and the photoproducts chemically labilized. The photofootprint of the *lac* repressor is then revealed by denaturing the DNA from both plasmids and comparing their strand breakage pattern on sequencing gels. As shown in Fig. 3, the binding of *lac* repressor *in vivo* enhances the formation of two photoproducts and inhibits the formation of seven others.

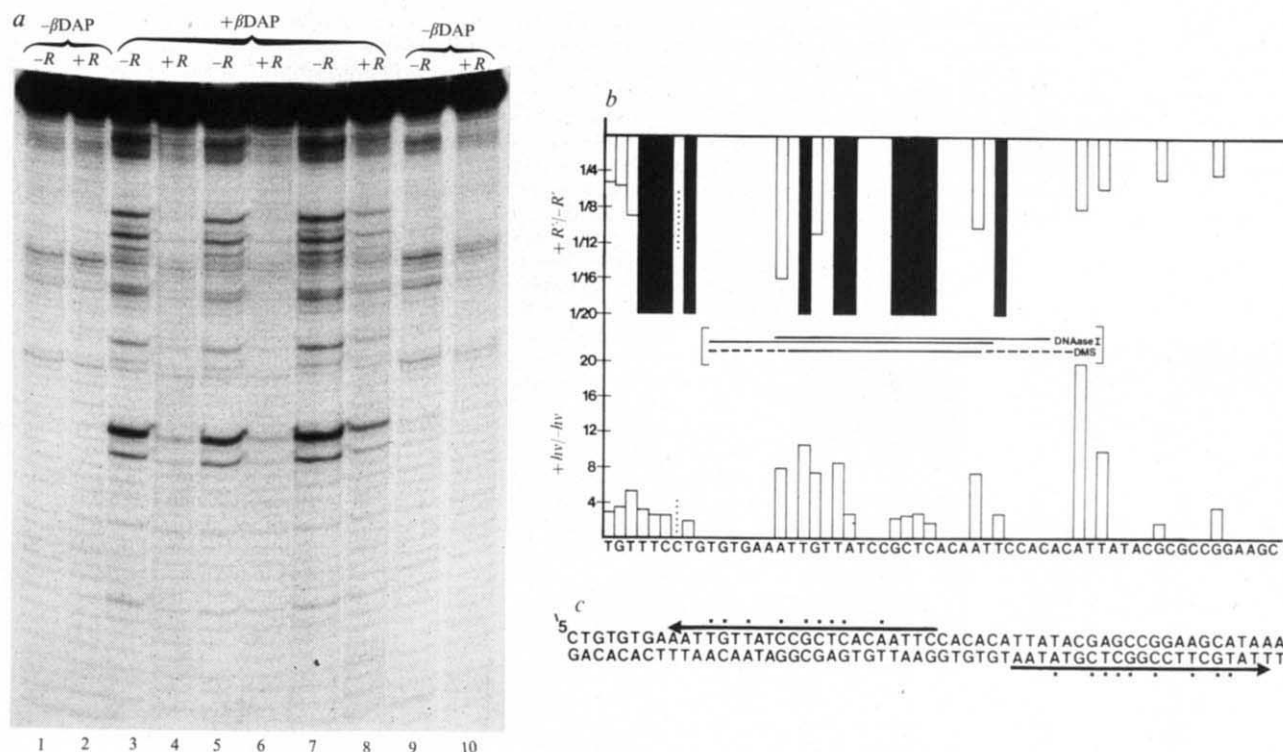


fig. 4 Photofootprints of the *lac* repressor obtained *in vitro* with a triplet sensitizer. **a**, An autoradiogram of the *lac* repressor–*lac* operator photofootprint. Lanes 1, 2, the effect of a 30-min irradiation of the *lac* operator at 315 nm in the absence of sensitizer. Lane 1 contains no *lac* repressor while lane 2 contains *lac* repressor. Lanes 3–8, the effects of irradiating the *lac* operator with 315 nm light in the presence of 10 mM sensitizer (β -dimethylaminopropiophenone, Aldrich). Irradiation was for 30 min (lanes 3, 4; similar results were obtained in 5 min), 60 min (lanes 5, 6) or 120 min (lanes 7, 8). Odd-numbered lanes contain no *lac* repressor while even-numbered lanes contain *lac* repressor. Lanes 9, 10, the effect of a 120-min irradiation on the *lac* operator at 315 nm in the absence of sensitizer. Lane 9 contains no *lac* repressor while lane 10 contains repressor. **b**, Results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The cleavage of DNA by sensitizer in the absence of *lac* repressor is expressed as the ratio $(+hv/-hv)$ while the effect of *lac* repressor on sensitizer-induced cleavage is expressed as the ratio $(+R'/-R')$ (see Fig. 1 for nomenclature). A base whose cleavage could not be accurately quantified by microdensitometry is denoted by a dotted line. Fading of the photofootprint at long irradiation times is probably due to damage of the complex by the 315 nm light. **c**, The plausible pseudooperator site. Homologous bases are denoted by a dot.

Methods: Irradiation was with the same apparatus as described for Fig. 1 except that a monochromator (Instruments SA, Inc., Model 1200-UV-H10) was placed between the water filter and the sample. The monochromator entrance and exit sites were 2 mm (15 nm bandwidth).

The experimental conditions and reagents were the same as for Fig. 1.

The *lac* repressor binding site detected in these measurements agrees well with the *in vitro* photofootprinting results as well as with the *in vitro* results obtained by other techniques^{19–21}. Thus, contacts between the *lac* repressor and its operator can readily be detected *in vivo* with light.

A triplet sensitizer and psoralen

Although light alone can be used to detect *lac* repressor and, as shown below, RNA polymerase binding, less tightly associated proteins might go undetected. We have therefore sought to develop additional photochemical techniques which may be more sensitive to these types of protein–DNA interactions. Two such techniques have been developed. In the first, a triplet sensitizer, β -dimethylaminopropiophenone (β DAP) is used to excite DNA indirectly. As demonstrated previously, when excited by 315 nm light *in vivo*, β DAP can transfer its excitation energy to DNA, causing the DNA to assume an excited triplet state²⁵. Because this triplet state yields many of the same photoproducts formed by direct UV irradiation of DNA, the chemical labilization procedure outlined here can be used to detect β DAP-induced photolesions. The major advantage of using a sensitizer to introduce photolesions into DNA is the requirement of a collision between sensitizer and DNA before the sensitizer can transfer its excitation energy to DNA²⁶. Thus, photoproduct formation induced by β DAP is expected to be more sensitive to protein binding than direct irradiation by UV light.

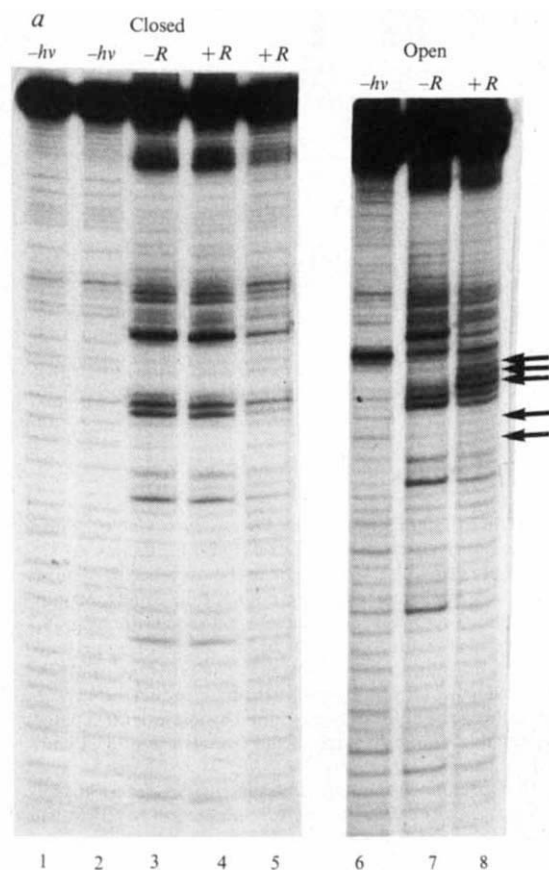
To test these hypotheses, the same *lac* repressor–*lac* operator experiment described in Fig. 1 was repeated using 315 nm light

to excite the sensitizer. As shown in Fig. 4, in the absence of sensitizer, DNA is undamaged by 315 nm radiation, but in the presence of sensitizer, irradiation introduces a larger number of photoproducts into the DNA. When these experiments are repeated in the presence of *lac* repressor, repressor binding strongly inhibits the formation of many of the β DAP-sensitized photoreactions.

The *lac* repressor photofootprint obtained with β DAP exhibits many of the same features observed by other techniques. For example, that region of the operator strongly footprinted by bromouracil cross-linking⁴, DNase I digestion²⁰ and dimethyl sulphate (DMS)¹⁹ is also strongly protected from β DAP-induced cleavage. However, in contrast to these measurements, β DAP also detects *lac* repressor contacts outside the presumed operator. Strong contacts are observed downstream from the operator while weaker ones are detected upstream. Although strong contacts downstream are not detected by bromouracil cross-linking or DMS experiments, reexamination of the DNase I results²⁰ reveals that *lac* repressor both enhances and inhibits DNase I cleavages in this region. As photofootprinting with UV light also detects repressor contacts in this region (see Fig. 1), strong inhibition of sensitizer-induced cleavage in this area probably results from repressor contacts that are insensitive to bromouracil cross-linking or DMS protection experiments.

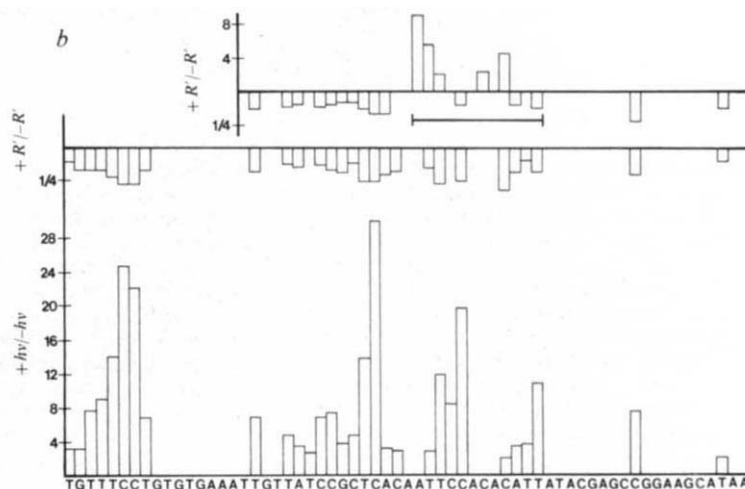
Weak contacts detected upstream from the presumed *lac* operator with β DAP are also detected by direct irradiation with UV light (see Fig. 1). Because some of these cleavages occur well upstream of the presumed operator, we have examined the sequence of this area for the possibility of a second or pseudo-operator binding site. As shown in Fig. 4, we indeed found a

Fig. 5 *In vivo* photofootprints of *E. coli* RNA polymerase bound to the *lac* UV-5 promoter in a closed and open complex. **a**, An autoradiogram of the RNA polymerase photofootprint. Lanes 1–5, photofootprints of the closed RNA polymerase complex; lanes 6–8, photofootprints of the open RNA polymerase complex. Lanes 1 and 2 are no light controls. Lane 1 contains no RNA polymerase whereas lane 2 contains 100 nM RNA polymerase. Lane 3 shows the effect of irradiating the UV-5 promoter at 16 °C in the absence of RNA polymerase. Lanes 4 and 5 show the effect of irradiating the UV-5 promoter at 16 °C in the presence of 50 and 100 nM RNA polymerase, respectively. Lane 6 is a no-light control for the open complex. The dark band approximately one-third of the way down from the top of the gel is a contaminating *EcoRI** band present in this preparation of DNA. It results from overdigesting the plasmid pJW231 with *EcoRI* and *Bam*HI to liberate the desired 95-bp UV-5 promoter fragment used in these photofootprinting experiments. Lane 7 shows the effect of irradiating the UV-5 promoter at 32 °C in the absence of RNA polymerase. Lane 8 shows the effect of irradiating the UV-5 promoter at 32 °C in the presence of 100 nM RNA polymerase. Arrows indicate the 5 bases showing the enhanced photoreactivity on open complex formation. **b**, Results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The effect of light on the cleavage of the UV-5 promoter in the absence of RNA polymerase is expressed as the ratio $(+hv/-hv)$ while the effect of RNA polymerase on light-induced cleavage of the UV-5 promoter is expressed as the ratio $(+R'/-R')$ (see Fig. 1 for nomenclature). Whereas RNA polymerase does not enhance cleavage of any bases at 16 °C, at 32 °C the enzyme enhances the cleavage of 5 bases. These enhanced cleavages occur over the region of the promoter known to be melted at the higher temperature (see bracketed line)^{24–26}.



polymerase. Arrows indicate the 5 bases showing the enhanced photoreactivity on open complex formation. **b**, Results of the autoradiogram quantified by microdensitometry and displayed as a histogram. The effect of light on the cleavage of the UV-5 promoter in the absence of RNA polymerase is expressed as the ratio $(+hv/-hv)$ while the effect of RNA polymerase on light-induced cleavage of the UV-5 promoter is expressed as the ratio $(+R'/-R')$ (see Fig. 1 for nomenclature). Whereas RNA polymerase does not enhance cleavage of any bases at 16 °C, at 32 °C the enzyme enhances the cleavage of 5 bases. These enhanced cleavages occur over the region of the promoter known to be melted at the higher temperature (see bracketed line)^{24–26}.

Methods: Irradiation was for 35 s in 200 μ l Micropets as described in Fig. 1 legend. In a typical experiment, 12 μ l of a solution consisting of 25 mM HEPES (pH 8.0), 100 mM KCl, 10 mM MgCl₂, 1 mM 2-mercaptoethanol, 100 μ g ml⁻¹ gelatin, 5% glycerol and 15 ng of the same 95-bp *lac* operator fragment used in Fig. 1, were incubated at 37 °C for 20 min and then irradiated in the absence or presence of RNA polymerase at 16 °C or 32 °C²⁵. The samples were then processed and analysed by the method described in Fig. 1 legend.



second potential operator sequence, identical with the *k* *lac* operator at 9 bases. The observed 9-bp homology makes this site a reasonable candidate for repressor binding because a known repressor binding site, just upstream from the 'cap' binding site of the *lac* operon, also exhibits a 9-bp homology with the *lac* operator²⁷. Binding studies of the *lac* repressor to a restriction fragment bearing both of these 9-bp operator sequences plus the known *lac* operator shows the formation of three strong complexes with relative affinities of 1, 1/200 and 1/800 (ref. 28). Although these measurements did not locate the weakest binding site, the affinity observed for this site is compatible with binding at the proposed site in Fig. 4, as the other 9-bp operator binding site exhibits only a fourfold greater affinity.

Although repressor binding at the proposed pseudo-operator sequence in Fig. 4 was not reported in DNase I protection experiments²⁰, reexamination of these results does reveal weak protection in this area. The failure of bromouracil cross-linking and DMS protection experiments to detect this repressor binding site may be due to the fact that a restriction fragment bearing only one-half of this operator was used in those experiments.

A second small organic molecule examined for its potential to footprint DNA photochemically is the well known intercalator psoralen. When excited by 350 nm radiation, psoralen covalently binds to DNA^{29,30}. Because covalent addition saturates the 5,6-double bond of pyrimidines³¹, the chemical labilization procedures used to detect UV and sensitizer-induced photoproducts can also be used to detect psoralen binding. A major advantage

Previous studies have shown that *in vivo* psoralen does not photoreact with nucleosome-bound DNA.^{32,33} We have determined that psoralen binding can be detected at the base pair level using the chemical degradation procedure and that such binding is sensitive to *lac* repressor binding. Experiments are being conducted to determine whether nucleosome binding can be detected with psoralen, UV light and β DAP.

Photofootprinting structural changes in DNA

Previous measurements have shown that UV-induced damage to DNA may be enhanced by structural changes in the DNA. For example, pyrimidine dimer formation is enhanced in single- versus double-stranded DNA⁸; apparently, geometrical changes necessary for dimer formation are easier in single-stranded DNA. We have examined the separation of DNA strands by RNA polymerase to establish whether light can be used to footprint structural changes in DNA.

At low temperatures, *E. coli* RNA polymerase forms a closed complex at promoters²³. When the temperature of this complex is raised, a short stretch of DNA is unpaired by RNA polymerase, resulting in the formation of a transcriptionally active open complex^{34–37}. Photofootprinting of both the open and closed complexes of RNA polymerase bound to the *lac* UV-5 promoter revealed that the formation of both complexes can readily be detected with UV light (Fig. 5), binding being characterized by reductions in photoproduct formation throughout the promoter. However, whereas the closed complex does not enhance the photoreactivity of any bases, the open complex

enhances the formation of five photoproducts. The location of these five enhanced photolesions is in excellent agreement with the known site of DNA strand separation determined by other measurements³⁵⁻³⁷. Thus, a small structural change in DNA, such as its unpairing by RNA polymerase, can readily be detected at the base pair level by light.

Conclusions

The photochemical approaches used in these experiments to footprint DNA have several advantages over other methods. First, since light readily permeates cells, DNA can be footprinted *in vivo*. Indeed, when combined with the procedure of sequencing genomic DNA directly by indirect end-labelling³⁸, it should be possible to footprint almost any gene *in vivo* with

light. Second, because the footprinting reaction is directly coupled to the absorption of light, it can be initiated essentially instantaneously and terminated within 10^{-9} to 10^{-6} s (the lifetime of the excited state). Thus, by using more powerful light sources it should be possible to footprint, kinetically, structural changes in DNA as well as protein movement along the DNA. Third, because the quantum yield of a photochemical reaction is relatively insensitive to changes in temperature or solvent, footprints of DNA can be obtained in a wide range of conditions. Fourth, because the chemical procedure developed to labilize DNA photoproducts is also expected to work on RNA, it should be possible to photofingerprint RNA as well as DNA.

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LETTERS TO NATURE

Search for excess cosmic-ray events from the galactic plane

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Wdowczyk and Wolfendale¹ have recently suggested that the cosmic-ray beam at 10^{15} - 10^{16} eV may contain a significant flux of γ rays from the direction of the galactic plane, and that this flux may affect the measured all-particle anisotropy and energy spectrum at these energies. We have searched for an excess of cosmic-ray events from the direction of the galactic plane. We can set upper limits which are close to the flux predicted by Wdowczyk and Wolfendale for the full galactic plane, and also for regions around the galactic centre. We have used these upper limits to place limits on the apparent brightness distribution of galactic γ -ray sources above 10^{15} eV, and on any contribution these sources can make to the cosmic-ray energy spectrum at the highest energies.

Recent observations using extensive air shower techniques have provided evidence for galactic sources²⁻⁶, and possibly extragalactic sources⁷, capable of producing γ rays with ultra high energies (10^{15} - 10^{16} eV). Wdowczyk and Wolfendale¹ have examined the possibility that such galactic γ rays might be responsible for structure found in the cosmic-ray energy spectrum and anisotropy at these energies, and concluded that there is some experimental evidence for this. No individual datum is conclusive but the cited evidence suggests that γ rays may be responsible for 1% of cosmic ray showers from the direction of

the galactic plane ($|b| < 10^\circ$), and a rather larger fraction from near the galactic centre. This would constitute $\sim 0.2\%$ of the primary cosmic radiation.

We have used data from 3yr of operation of the University of Adelaide extensive air shower array at Buckland Park⁸ (1.3×10^5 events with a mean energy of $\sim 5 \times 10^{15}$ eV) to search for any excess from the galactic plane. We summed all events observed with galactic latitude ($|b|$) below $\sim 10^\circ$ and compared this sum with that expected on the basis of events observed outside the galactic plane but at similar declinations. The data from Buckland Park are presented in Table 1 together with data from three other experiments⁹⁻¹¹ operating in a similar energy range. This then gives a full, though non-uniform, coverage of the galactic plane. The data from the experiments by Clark⁹ and Chitnis *et al.*¹⁰ were extracted from published celestial intensity maps. The data from Haverah Park were obtained with their 50-m system and were extracted from ref. 11. In the latter cases, the original investigators have presented their own null results for $|b| < 20^\circ$ (ref. 9), $|b| < 10^\circ$ (ref. 10) and $|b| < 5^\circ$ (refs 11, 12). The Haverah Park and MIT experiments observed essentially the same region of sky. Data from these two experiments have been combined and are also shown in Table 1, clearly there is no evidence for there being any enhancement for $|b| \leq 10^\circ$.

In estimating any upper limits to a γ -ray contribution from the galactic plane, one must take into account the statistical uncertainty in the cosmic-ray background estimation¹³. This depends on the total number of events used to estimate the background. The ratio, α , of the estimated background counts for $|b| \leq 10^\circ$ to the total number of events with $|b| \geq 10^\circ$ which were used to estimate the background is given in Table 1 for each experiment. Recently, Protheroe¹⁴ has derived 95% (~ 1.65 s.d.) upper limits to the ratio of the source contribution to the background contribution given various observed counts,

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Table 1 Observed and expected number of events from $|b| \leq 10^\circ$ for various ranges of galactic longitude

Experiment	Galactic plane					Galactic centre region	
	Buckland Park 35° S	Kodaikanal 10° N	MIT 42.5° N	Haverah Park 54° N	Haverah Park + MIT —	Buckland Park	Buckland Park
Ref.	This work	10	9	11	9, 11		
Approximate range of galactic long.	210°–30°	$\begin{Bmatrix} 0^\circ\text{--}80^\circ \\ 170^\circ\text{--}240^\circ \end{Bmatrix}$	40°–200°	40°–200°	40°–200°	350°–10°	300°–60°
No. observed	21156	13,555	552	99,408	99,960	4,631	10,221
Estimated background	21103	13,529	530	99,421	99,951	4,726	10,258
α	0.191	0.184	0.251	0.276	0.276	0.0833	0.0846
γ -ray/cosmic ray upper limits (%)							
84%	1.2	1.5	10.0	0.50	0.51	1.3	1.3
95%	1.7	2.0	13.0	0.70	0.71	2.0	1.8

84% and 95% confidence upper limits are given for the ratio of γ -ray flux to cosmic-ray flux.

estimated background, and α values. The procedure involved estimating the joint probability density function of the true background having some value and the ratio of source contribution and background having some value, and is described fully in ref. 14. The 95% confidence upper limit to the ratio of γ -ray contribution to cosmic-ray contribution for $|b| \leq 10^\circ$ has been calculated for each experiment using this procedure and is given in Table 1 which also shows, 84% (~ 1 s.d.) upper limits calculated in the same way. Only the Haverah Park data have a 95% confidence upper limit to a galactic plane γ -ray contribution which is below $\sim 1\%$ of the cosmic-ray contribution.

By taking the lowest upper limit from the experiments which cover a particular region of the galactic plane and integrating over galactic longitude, we can obtain an estimate of the upper limit to any γ -ray enhancement for the galactic plane as a whole. Doing this, we obtain an 84% confidence upper limit of $\sim 0.9\%$, and a 95% confidence upper limit of $\sim 1.3\%$, for any γ -ray enhancement from the whole galactic plane ($|b| \leq 10^\circ$). These upper limits are close to the $\sim 1\%$ enhancement predicted by Wdowczyk and Wolfendale¹.

The galactic centre passes almost overhead at Adelaide. We have used our data to search for any enhancement from the galactic plane within 60° of the galactic centre. It is possible that the galactic centre has a more localized excess and we have also examined events from the direction of the galactic centre (within $\sim 10^\circ$). The results are also given in Table 1 together with the upper limits we derive for any γ -ray enhancement from these regions of the sky. The data show no significant enhancement over the expected background. Again, however, the 95% upper limits are very close to the flux predicted by Wdowczyk and Wolfendale¹.

At 10^{15} – 10^{16} eV, the overall cosmic-ray anisotropy has a first harmonic amplitude of $\sim 0.3\%$ (ref. 1). Our limits on any excess flux from the galactic plane appear to preclude the possibility that such a high amplitude for a whole sky anisotropy could result solely from galactic γ rays.

There is no doubt that an excess of γ -ray events should exist from the galactic plane due to known galactic ultra high energy γ -ray sources. None of these sources has, however, been associated with large numbers of γ rays arriving at the Earth. The γ -ray sources which have been observed each contribute $\sim 0.1\%$ of the total cosmic ray events detected from the galactic plane by each experiment. Our 95% confidence upper limit of 1.3% for the total γ -ray excess from the galactic plane thus corresponds to only ~ 40 times the observed flux from a single known source.

Cygnus X-3 (refs 2, 3) is one of the brightest and also the most distant of the known galactic sources, being at a distance of the order of the galactic radius. This suggests that a simple disk population brightness, S , distribution (that is $N(>S) \propto S^{-1}$) is unlikely to be valid, and that instead the distribution of observed fluxes reflects the (unknown) γ -ray luminosity function of the sources.

Cygnus X-3 is not in the direction of the galactic centre. Neither are the Crab pulsar^{5,6} and Vela X-1 (ref. 4), the other galactic ultra high energy candidate sources. Our upper limits on the inner galaxy flux ($|b| < 10^\circ$, $|l| < 60^\circ$) will not allow an enhancement above the total flux of outer galaxy sources such as is found for X rays (9.4×10^{15}) or γ rays above 100 MeV (2.2×10^{15}) even if these three sources were the only sources contributing to the 10^{15} – 10^{16} eV γ -ray flux from the outer galaxy.

We note that if the total flux of γ rays were lower by about 10 times than that postulated by Wdowczyk and Wolfendale (it cannot be much lower than this on the basis of known sources) and their spectrum extends to $\geq 10^{19}$ eV, these events would distort the observed cosmic ray spectrum and produce a flattening at $\sim 10^{18}$ eV. With some steepening in the γ -ray spectrum above 10^{16} eV they would instead produce an 'ankle' in the cosmic ray spectrum as observed¹⁶ at 10^{19} eV. It may then not be necessary to postulate a sharp cutoff to the γ -ray spectrum at 10^{17} – 10^{18} eV which Wdowczyk and Wolfendale¹ needed to avoid gross disagreement with the observed cosmic-ray spectrum. Support for this suggestion may be found in data for energies above 10^{19} eV presented by the Sydney group¹⁷. These data show an excess from the galactic plane for showers with energies above 4×10^{19} eV (six events are observed for $|b| < 10^\circ$ where we expect 1.0 ± 0.5). In this case, the Sydney detectors would be predominantly detecting muons from photoproduced pions^{18–19}. At these energies the flux of such muons is expected to be quite significant for γ -ray initiated showers.

We have set a 95% confidence upper limit of 1.3% for any enhancement by γ rays of the 10^{15} – 10^{16} eV cosmic ray flux from the galactic plane. This sets a limit to the total flux of ultra high energy galactic γ rays at ~ 40 times that observed from Cygnus X-3. Cygnus X-3 must then be amongst the brightest sources of such γ rays in our galaxy. These γ rays may be responsible for the ankle in the cosmic-ray energy spectrum above 10^{19} eV.

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Radio wave scattering in the galactic disk

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Electron density turbulence in the Galaxy causes radio sources to be broadened angularly by amounts that depend strongly on the direction of the source. Recent scattering measurements using very long baseline interferometry (VLBI)¹ and interplanetary scintillations (IPS)² of extragalactic sources, and interstellar scintillations (ISS) of pulsars³ are used here to constrain the distribution of scattering material in the Galaxy. Although ubiquitous, scattering material appears to be highly concentrated in a thin disk of ~ 100 pc thickness and in clumps of 1–10 pc size probably associated with H II regions, stellar wind bubbles, and/or supernova shocks. Typical transverse velocities of scattering material are much smaller than typical pulsar velocities. We predict the scattering angle as a function of galactic latitude. The results imply a maximum observable surface brightness for incoherent sources of electron synchrotron radiation.

Recent work has demonstrated that electron density fluctuations on length scales of 10^9 – 10^{11} cm conform to a power law wavenumber spectrum of the form $C_n^2 q^{-\alpha}$ with $\alpha = 3.6 \pm 0.2$ (refs 3–6) where q is the wavenumber. This form of the spectrum is consistent with detailed measurements on a few lines-of-sight. Less detailed measurements on others sometimes suggest different values of α (refs 3, 7). We adopt a specific value ($\alpha = 11/3$) in the analysis below which is in agreement with the best studied lines-of-sight. Variations of α with direction will not grossly modify any of our calculations. There is some evidence that the spectrum extends over six or more decades in wavenumber, q (ref. 6). Although the slope of the spectrum shows little variation, the level of turbulence C_n^2 varies by at least four decades, both in going from high to low galactic latitudes and between lines-of-sight confined to the galactic disk³.

We now use scattering and scintillation observables to constrain further the distribution of scattering material in the Galaxy and to provide a model for the observed scattering disk size as a function of galactic coordinates. VLBI measurements determine the scattering angle θ_{ISS} (FWHM) directly. IPS yields angular sizes by comparing the scintillation power spectrum of a source seen through the interplanetary medium with that of a point calibrating source at the same solar elongation. Interstellar scintillations of pulsars are parameterized by the scintillation bandwidth Δf_{ISS} and scintillation fluctuation time Δt_{ISS} (both half widths at half maxima of the two-dimensional intensity correlation function). The scattering angle for a plane-wave source is^{3,8} (with C_n^2 in units of $\text{m}^{-20/3}$)

$$\theta_{\text{ISS}} \approx 0.133 (L_{\text{kpc}} \langle C_n^2 \rangle)^{3/5} f_{\text{GHz}}^{-11/5} \text{ arc s} \quad (1)$$

where L is the effective path length in kpc, f is the observation frequency in GHz, and $\langle \rangle$ denotes average over path length. The wavenumber that contributes most to the scattering of a given source (if all turbulence were confined to a thin layer at distance $L/2$) is $q_{\text{eff}} \approx 2\pi\theta_{\text{ISS}}/\lambda$. Relative motion of source, turbulence and observer causes time variations with an effective velocity.

$$V_{\text{ISS}} \approx 10^{4.4} (L_{\text{kpc}} \Delta f_{\text{ISS}})^{1/2} / \Delta t_{\text{ISS}} f_{\text{GHz}} \text{ km s}^{-1} \quad (2)$$

(Δf_{ISS} in MHz) which has been shown to correlate at the 66% level with pulsar velocities obtained interferometrically⁹. Consequently it appears that V_{ISS} is dominated by the pulsar velocity rather than that of the scattering medium. This would seem to rule out enhanced ISS from stellar winds themselves which, by

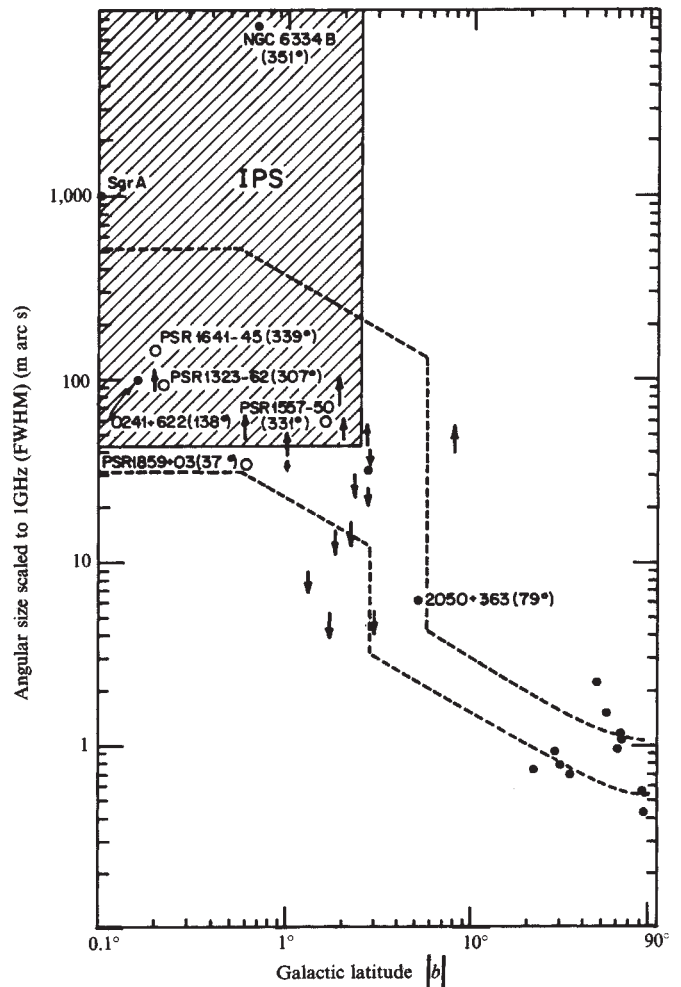


Fig. 1 Scattering diameter at 1 GHz versus galactic latitude for the inner galaxy. ●, VLBI measurements of extragalactic sources^{1,26,27} and arrows represent lower and upper limits. The point for NGC 6334 B is an extragalactic source viewed through the H II complex NGC 6334 (ref. 28). ○, Implied angles derived from pulsar scintillation measurements and the effective scattering angle for a source of plane waves at the distance of the pulsar (5–10 kpc). The shaded region summarizes the IPS observations of sources in the direction of the galactic centre² and the dashed lines are predicted angles from a survey of pulsar ISS³.

analogy with IPS and the solar wind, would yield $V_{\text{ISS}} \sim 10^2$ – 10^3 km s⁻¹.

Pulsar ISS measurements for 76 objects suggest a two-component model for C_n^2 : component A with scale height $H_A \geq 0.5$ kpc, $C_n^2 \sim 10^{-3.5}$, and volume filling factor ~ 1 ; component B wherein C_n^2 is large in clump-like regions of size $R_{\text{clump}} \sim 1$ – 10 pc with very small filling factor $\sim 10^{-4}$ – 10^{-3} , and small scale height, $H_B \sim 50$ – 100 pc (ref. 3). The pathlength-averaged C_n^2 , $\langle C_n^2 \rangle$, increases systematically with decreasing galactocentric radius, suggestive of component B clumps being more common at galactocentric radii smaller than that of the Sun. However, the increase may also reflect an increasing probability for encountering clumps with increasingly larger internal C_n^2 . The anticentre directions yield $\langle C_n^2 \rangle$ that are typically smaller than those within the solar circle for the same distance, so that there seems to be a real galactocentric radial dependence. The mean free path for encountering clumps is $L_{\text{mfp}} \sim 2$ – 10 kpc so lines of sight with $|b| \geq 10^\circ$ sample only the 'A' component.

The pulsar results are in good agreement with both recent VLBI and IPS measurements. A recent survey¹ of low-latitude ($|b| < 5^\circ$) compact extragalactic radio sources at 408 MHz shows that 8 out of 18 sources towards the inner Galaxy ($10^\circ \leq l < 80^\circ$)

are sufficiently scattered to yield no fringes implying, typically, $\theta \geq 0.2$ – 0.3 arc s. By comparison, all 11 sources within $197^\circ < l < 234^\circ$ had $\theta < 0.2$ arc s and only one showed no fringes, probably because it lies behind an H II region. The VLBI results are consistent with a 50% probability for heavy scattering of extragalactic sources in directions $10^\circ \leq l \leq 80^\circ$ and $|b| \leq 1^\circ$. A search for sources that display IPS² at 327 MHz in the regions $-10^\circ < l < 30^\circ$ and $180^\circ < l < 210^\circ$ with $|b| < 12^\circ$ show a marked lack of scintillating sources towards the inner Galaxy. Only two out of an expected eight such sources were detected above a flux density of 0.2 Jy for $|b| < 2.5^\circ$, suggesting that the scattering disk due to interstellar scattering ≥ 0.5 arc s, typically.

We combine all data for the inner galaxy in Fig. 1, which shows the equivalent scattering angle (FWHM) at 1 GHz after scaling all measurements according to $\lambda^{2.2}$. VLBI measurements, upper limits, and lower limits are shown as dots or arrows, the IPS results are summarized as a shaded region which designates $\theta > 43$ m arc s for $|b| < 2.5^\circ$, and the pulsar measurements are displayed as predictions for the scattering angle derived from the two-component model. We also show the implied scattering angles for four highly scattered pulsars. Two prediction curves are shown which bracket the expected range of angles. For $|b| \geq 3^\circ$ – 6° , scattering angles are determined by the 'A' medium for nearly all lines-of-sight and are deterministic. For smaller latitudes the scattering angle is a random variable (associated with the probability of intersecting a component B clump) and may fall outside the predicted range, as some of the upper limits apparently do. Break points in the prediction curves occur at angles $\sim H_B/L_{\max}$ and $H_A/L_{\max} \sim H_B/L_{\text{mfp}}$ where $L_{\max}$ is the maximum possible pathlength, taken to be 10 kpc. Apart from the break points and the constant parts of the curves for $|b| \leq 0.7^\circ$, the scattering angle varies as $\sim |\sin b|^{-3/5}$ but with different proportionality constants for different ranges of b . We note that our results for $|b| \geq 10^\circ$ agree with previous results based solely on IPS measurements^{10,11}.

The predicted scattering angle can be considered the apparent diameter of a scattered point source only as long as the condition of strong scattering holds⁸. Roughly, this is true if $q_{\text{eff}} \geq q_{\text{Fresnel}} = 2\pi/\sqrt{\lambda L}$ or $\theta_{\text{ISS}} \geq \sqrt{\lambda/L}$. At smaller λ , the scattered source brightness distribution takes on a core-halo form (for intrinsic source size $\ll$ scattering disk size). At $b = 90^\circ$, $\theta_{\text{ISS}} \sim 1$ m arc s at 1 GHz implies that strong scattering occurs for $\lambda \geq \lambda_c \sim 4$ cm. At directions towards the inner Galaxy with $|b| \leq 1^\circ$, $\lambda_c \sim 0.12$ cm for $\theta_{\text{ISS}} \sim 100$ m arc s and an effective screen distance of 5 kpc. The compact source Sgr A at the galactic centre with $\theta_{\text{ISS}} \sim 1$ arc s at 1 GHz (ref. 12) yields $\lambda_c \sim 0.3$ mm if the apparent source size at decimetric wavelengths is indeed due to scattering. Although free-free absorption¹³ from gas with a density $\propto r^{-1}$ can also produce a wavelength dependence $\theta \propto \lambda^2$ and the angular size is an order of magnitude larger than the most scattered pulsar (PSR1641–45), the necessary amount of scattering (from the general galactic turbulence as opposed to turbulence local to the source) is not an unrealistic extrapolation of the pulsar results. This follows because Sgr A is further than the most scattered pulsar and the line-of-sight passes through much more ionized material. The pulsar results themselves indicate that θ_{ISS} increases much more rapidly than the $L^{3/5}$ variation that would be expected for a medium with a uniform level of turbulence. We also note that scattering may produce asymmetric images such as has been reported for Sgr A¹⁴. Turbulence in an anisotropic medium, that is, one with a preferred direction associated with a velocity flow, magnetic field or gradient in the level of turbulence, has an anisotropic wavenumber spectrum which will yield an asymmetric scattering disk. As yet there is no evidence for such an asymmetry from observations of astronomical sources. IPS measurements at 74 MHz (ref. 15) show asymmetric diffraction patterns which are thought to derive from the intrinsic source structure of extragalactic sources because the Crab pulsar, after angular broadening due to ISS, shows no asymmetries in its IPS diffraction. The possibility still remains, however, that sources showing large amounts of scattering will be broadened asymmetrically.

The available evidence implies that scattering occurs along every line-of-sight, albeit by vastly different amounts. Turbulence extends to at least 0.5 kpc in a diffuse component but has large amplitudes in clumps whose distribution resembles a population I class of objects. Pulsars that are known to have intervening H II regions (PSR0611+22) or supernova remnants (Crab and Vela) all show enhanced scattering. However, there is no knowledge of the relative importance of a large mean electron density and the energization required to drive the turbulence. What analysis exists implies that considerable energy is required to maintain the density fluctuations¹⁶.

Because of its ubiquity, ISS has important consequences concerning measurements of intrinsic source sizes, ψ . In the conditions $\theta_{\text{ISS}} \geq \psi \geq \zeta\theta_c$ where $\zeta \geq 1$ is a dimensionless numerical factor and $\theta_c \sim \lambda/2\pi L\theta_{\text{ISS}}$ is a critical angular size, the brightness distribution is irretrievably broadened into a distribution of width θ_{ISS} ^{17,18}. Only for $\psi \leq \theta_c$ is the radiation field coherent over the scattering disk, a requirement for observing fully modulated interstellar scintillations and for determining ψ from interferometric observations, provided the bandwidth and integration time are less than those characterizing the ISS (for example, Δf_{ISS} and Δt_{ISS} defined above)^{17,18}. ISS observations can probe intrinsic sizes with $\zeta = \psi/\theta_c \leq 10^3$ by measuring intensity variations down to a modulation index $m_{\text{ISS}} \sim \zeta^{-1} \sim 0.1\%$ (smaller indices are probably impossible to measure because of instrumental effects^{19–23} and therefore $\psi \gg \theta_c$ for these sources). (Recent work²⁴ has suggested that intensity variations of extragalactic sources may be caused by refraction from galactic electron density turbulence. This possibility is peripheral to the issues we discuss here.) Although new classes of extragalactic objects may be discovered which satisfy $\psi \leq \theta_c$, at present it seems that $\psi \gg \theta_c$ for extragalactic sources and therefore any interferometer observation will measure an angular size θ_{ISS} if $\psi < \theta_{\text{ISS}}$ and $\lambda > \lambda_c \sim 4$ cm (observing perpendicular to the galactic plane). Therefore, interferometer observations with baselines $\sim 10 R_{\text{Earth}} f_{\text{GHz}}^{1.2}$ will tend to be dominated by ISS, and baselines twice this size will totally resolve the scattering disk (for $f \leq 7$ GHz or $\lambda > \lambda_c \sim 4$ cm). Taking $\theta_{\text{ISS}} = 0.5$ – 2.0 m arc s at 1 GHz, we find that the maximum observable brightness temperature for high-latitude sources is

$$T_{\text{b max}} \approx 10^{12.1 \pm 0.6} S_{Jy} f_{\text{GHz}}^{2.4} \text{ K}, f \leq 7 \text{ GHz} \quad (3)$$

Therefore, intrinsic source structure having a brightness temperature close to or in excess of the inverse Compton limit ($\sim 10^{12}$ K) will be difficult, if not impossible, to resolve at frequencies < 1 GHz. We point out, however, that the detection of diffraction-produced intensity variations at sub-GHz frequencies would necessarily imply $T_{\text{b}} > 10^{12}$ K (refs 19–21).

The only known class of sources for which these limitations can be overcome are pulsars, which show interstellar scintillations and whose emission regions satisfy $\psi < \theta_c$ (ref. 25). VLBI observations with bandwidth and integration time smaller than Δf_{ISS} and Δt_{ISS} , respectively, will yield fringes on baselines $\leq \lambda/\psi$ whose phase and amplitude vary randomly on a time scale Δt_{ISS} . Consequently, fringes should be detectable out to at least 1 AU for a typical pulsar at 1 kpc distance and probably on much longer baselines because emission regions are evidently much smaller than the light cylinder radius²⁵.

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Hercules X-1—a 1,000 GeV γ -ray pulsar

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An X-ray pulsar has been detected for the first time as a very high-energy (VHE) γ -ray pulsar. X-ray emission from Hercules X-1 is multiply periodic with a pulsar period of 1.24 s, a binary orbital period of 1.7 day and a 35-day amplitude modulation of unknown origin characterized by a sharp turn-on followed by a slow decline over 11 days. During a drift-scan we have detected a significant 3-min outburst of VHE γ rays, which had a 1.24-s periodicity. This outburst occurred 35 days before an observed X-ray turn-on. The γ -ray luminosity during the outburst was of the same order as the X-ray luminosity. Together with the coincidence in time, this suggests a connection between the mechanisms giving rise to the γ -ray outburst and the X-ray turn-on. Subsequent monitoring of the source during a period when the X-ray flux was decreasing yielded some evidence of much weaker pulsed γ -ray activity at the pulsar period. We note a possible similarity between this system and Cygnus X-3.

The discovery of Her X-1 as a pulsing (1.24-s period), eclipsing binary X-ray source was made by the UHURU group¹. The optical counterpart was identified as Hz Herculis² following an improved positional determination for the X-ray source and the detection of a related 1.7-day orbital period.

Studies^{3,4} of this first binary X-ray source to be detected reveal that the emission from the system is complex but involves variable emission of X-ray and optical photons with modulations, differing with wavelength, on time scales of 1.24 s, 1.7 and 35 days, the first two being attributed to the pulsar and binary orbit respectively. The origin of the 35-day modulation is less clear. It has been related to the 1.7-day orbital period⁵, although many theories have been based on the precession of the rotation axis or the obscuration of our view by a periodically moving disk. The pulsar period has been observed to spin up at a rate, averaged over 11 yr, of about 10^{-13} s s⁻¹. Recent measurements of the X-ray pulse period have been made by the Hackucho and Tenma satellites⁶. In two observations in June 1982 and May 1983, the X-ray data show periods of 1.2377870 s and 1.23778873 s respectively, agreeing broadly with the long-term trend for the pulsar to spin up, but with large and opposite signs of period derivative: $(+7.4 \pm 5.9) \times 10^{-13}$ s s⁻¹ and $(-3.6 \pm 1.3) \times 10^{-13}$ s s⁻¹ respectively. Optical and IR measurements⁷ made in July 1982, a date between the X-ray observations, suggested that the period could be much longer, perhaps as long as that noted in 1972. There is thus recent evidence for a large excursion of the pulsar period from the long-term trend, which is consistent with the indications for the behaviour in 1982–83 suggested by the signs of the X-ray period derivatives. Data on the pulsar period for the past 11 yr are summarized in Fig. 1.

Observations of Her X-1 were made on 17 April 1983 using the array of four telescopes of the Dugway VHE γ -ray facility^{8,9}. The telescopes have an energy threshold of $\sim 1,000$ GeV, an aperture of $\sim 2^\circ$ FWHM and a sensitivity (for a 3σ detection of pulsed emission lasting for 10 min) of $\sim 3 \times 10^{-10}$ cm⁻² s⁻¹. The initial observation comprised four drift scans, each lasting 40 min. The time of arrival of each recorded light flash was corrected to the Solar System barycentre. Assuming that the source of any γ rays is located at the same part of the system as the X-ray source, the times were corrected to the binary focus using the X-ray orbital ephemeris¹⁰.

Emission of VHE γ rays from Her X-1 was noted in the April observation as a marked outburst in the count rates of our telescopes during one of the four drift scans, which were taken in ideal sky and stable experimental conditions. The excess for the total time that Her X-1 was in the field of view of the telescope was $14 \pm 7\%$. The data indicated that the activity lasted for ~ 3 min, during which an enhancement of $33 \pm 10\%$ in the gross count rate occurred (see Fig. 2a). The events during this outburst were periodic with the characteristic X-ray pulsar period (Fig. 2b). Because the expected light curve is unknown at these energies, the general Rayleigh test⁸ for the strength of the first harmonic of a periodicity was used. The inherent sampling resolution of the periodicity is ~ 3 ms, rendering redundant the reduction of the orbit to the focus and preventing the measurement of an accurate period. The probability of this periodicity arising by chance is 4×10^{-4} , with an estimated first harmonic periodic content of 39%. The excess periodic γ -ray counts detected in the drift scan are therefore practically 100% pulsed. The profile of the pulsed component through the scan is depicted in Fig. 2c as the per cent pulsed in 3-min sliding sections. (The apparent narrowness of the peak in Fig. 2c compared with that in Fig. 2a is due to the nonlinear relation between chance probability and per cent pulsed signal.)

The increase in count rate and periodicity were shown both by those events triggering a single telescope, and independently by those events in which two telescopes detected the Cerenkov light pool from the same (perhaps higher energy) primary. The excess occurred ~ 5 min before the object was in the centre of the field of view of the telescope. Inter-detector fast timing indicates that the pulsed events in the excess arrive from a direction $\sim 1^\circ$ below the centre of the field of view coinciding with the position of Her X-1. There is therefore, in one of four drift-scans, a $>3\sigma$ count rate excess for 3 min from Her X-1, which is periodic at a $>3\sigma$ level at the X-ray periodic. Allowing for the degrees of freedom, the overall statistical significance of this single observation is 7×10^{-5} . The light curve for those events in the 3 min of activity is shown in Fig. 3 and has the

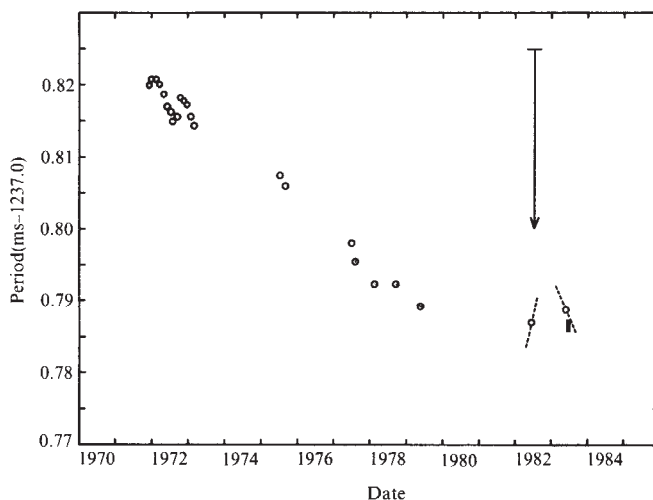


Fig. 1 The period of the Her X-1 pulsar. ■, Present value derived from the July observation; earlier values are from refs 6, 7. Dashed lines indicate the measured period derivative in X-ray observations.

characteristic broad single peak seen in X rays¹¹ (with a width about 30%). The peak γ -ray flux during the burst of activity is $1.2 \times 10^{-9} \text{ cm}^{-2} \text{ s}^{-1}$. The outburst occurred at phase 0.76 in the 1.7-day period and 35 days before a detected turn-on in X rays⁶.

Following the 17 April 1983 outburst, Her X-1 was tracked during 3–13 July 1983 and 7–11 October 1983. No further large count-rate excess in a 3-min interval similar to the April event

was seen. The July data show an overall periodicity at the 2σ level. Although contributing little to the statistical significance of the detection, these data taken over a prolonged interval allow a test of the accurate Tenma period. The 2σ effect was noted within the sampling range of the extrapolated period from the 1983 May Tenma X-ray measurement (1.2377873 s) and the detected period is indicated on Fig. 1. The time-averaged flux of γ rays is $0.84 \pm 0.4\%$ of the background cosmic ray flux, corresponding to a flux of $(3 \pm 1.5) \times 10^{-11} \text{ cm}^{-2} \text{ s}^{-1}$ for energy $> 1,000 \text{ GeV}$.

We conclude that Her X-1 is emitting pulsed VHE γ rays sporadically with chance probability of 7×10^{-5} and a peak flux of $1.2 \times 10^{-9} \text{ cm}^{-2} \text{ s}^{-1}$ above an energy of 1,000 GeV at a time in April 1983 when, according to the Tenma satellite data on 22 May 1983, the X-ray turn-on in the 35-day cycle may reliably be expected. The short outburst occurred at phase 0.76 in the 1.7-day orbit when the pulsar in the binary system was moving almost directly away from the Earth. X-ray turn-on has been observed to occur around phase 0.2 or 0.7 in the 1.7-day cycle every 35 days. The optical emission is also strongest at these same two phases¹². The shortest known time for turn-on is $< 700 \text{ s}$, being the interval between successive X-ray observations. It is thus possible that the X-ray turn-on is accompanied by the emission of a burst of VHE γ rays lasting $\sim 3 \text{ min}$. If this were the case, an X-ray turn-on took place on 17 April 1983 08.44 UT.

The July 1983 data indicate at the 2σ level that a time-averaged flux of $3 \times 10^{-11} \text{ cm}^{-2} \text{ s}^{-1}$ exists at the precise pulsar period according to a contemporaneous X-ray measurement (1.2377873 s), if we assume that the VHE γ rays originate with the X rays and make the appropriate allowances for the 1.7-day orbit. A conservative estimate of the probability of chance occurrence of the April event and the overall July emission is 5×10^{-5} .

The VHE γ -ray data were taken when unusual behaviour of the temporal features of the X-ray and optical components was reported^{5,7} and when the X-ray flux was decreasing. The time-averaged VHE γ -ray luminosity is $2 \times 10^{35} \text{ erg s}^{-1}$ (assuming a differential spectral slope of -3 and distance 5 kpc), which is $\sim 0.5\%$ of the X-ray luminosity including soft X rays.

We know of no previous attempt to detect VHE γ rays from this object. Flux limits¹³ on other X-ray sources suggest typical limits of $10^{-10} \text{ cm}^{-2} \text{ s}^{-1}$ at an energy of $\sim 1,000 \text{ GeV}$. The only other (and much more distant) X-ray binary source to be detected in VHE γ rays is Cygnus X-3. The emission from Cyg X-3 peaks at $10^{-9} \text{ cm}^{-2} \text{ s}^{-1}$ during short intervals of the 4.8-h binary period. The duration of the intervals of emission detected during drift scans vary from 15–30 min (ref. 14) to 1 h (ref. 15) (although these durations may be maximum values as they are similar to the duration of the drift scans) and they occur about 0.3 of a 4.8-h period before the X-ray minimum. Continuous observation of the object suggest that the emission may be as short as 3 min (ref. 9). In addition there have been indications that the strength of this 4.8-h periodic emission in both X rays and VHE γ rays

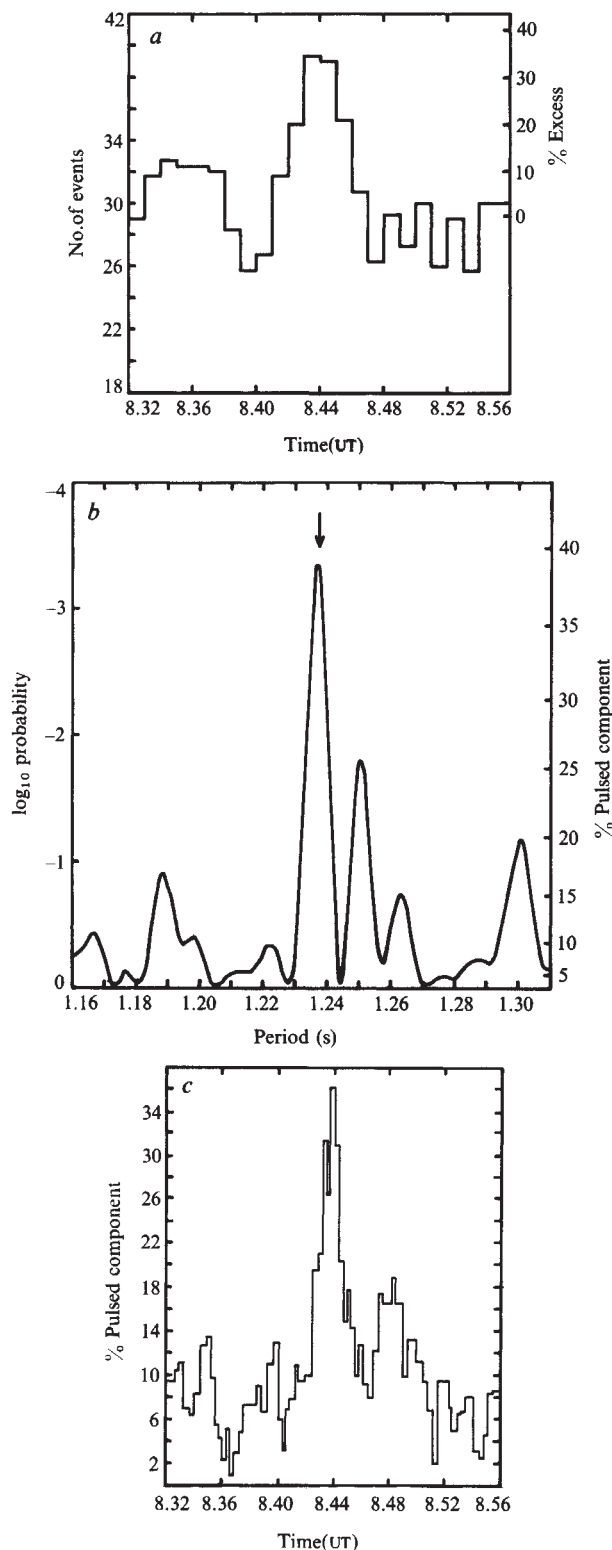


Fig. 2 *a*, The profile of the count rate per minute averaged over 3-min intervals during the observation on 17 April 1983. *b*, The probability of chance occurrence, using the Rayleigh test, and the first harmonic of the pulsed component of the events in the 3-min burst. The arrow marks the predicted period⁶. *c*, The profile of the periodicity (trial period 1.237 s) during the drift scan.

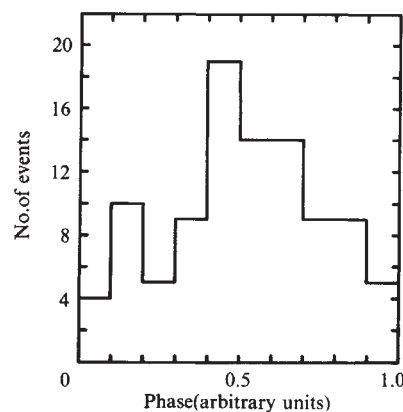


Fig. 3 The light curve for the VHE γ rays from Her X-1 using the trial period of 1.237 s. The data are from the 3 min of the outburst on 17 April 1983.

is itself modulated with a 34.1-day period. There is thus a resemblance between the VHE γ -ray properties of Cyg X-3 and Her X-1, although a pulsar period remains to be detected for Cyg X-3.

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Extraterrestrial platinum group nuggets in deep-sea sediments

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Iron cosmic spheres, composed of iron oxides surrounding a FeNi metal core, were discovered more than a century ago during the voyage of the HMS *Challenger*¹. That the spheres originated by atmospheric melting of meteoritic metal is supported by a variety of evidence, including elemental composition², the presence of the metastable oxide wustite³, similarity to analogues produced in the laboratory⁴ and content of ⁵³Mn produced by exposure to cosmic rays⁵. We report here a previously unrecognized property of the spheres. The most common spheres larger than 300 μm do not, in fact, contain FeNi metal cores, but instead contain a micrometre-sized nugget composed almost entirely of platinum group elements. These elements appear to have been concentrated by the oxidation of molten meteoritic metal during atmospheric entry. This process is critically dependent on the relative abundance of oxygen in the atmosphere, and the first appearance of the nuggets in the geological record may provide a marker indicating when the oxygen abundance attained half of its present level.

The spheres used in this study were collected with a magnetic collection sled (the Cosmic Muck Rake), towed across two locations on the ocean floor 1,000 km east of Hawaii⁶. The platinum group nuggets (PGN) were first identified as 5–10- μm diameter spheres in sectioned iron spheres (Fig. 1). A survey of 110 such polished sections, 300–500 μm in diameter, revealed four PGNs. We then mounted 11 300- μm iron spheres and sequentially polished completely through them, inspecting them at 1–2 μm depth intervals. Solitary PGNs were identified in seven of the spheres; additional nuggets may have been plucked out during polishing. None of the PGN-bearing spheres contained FeNi metal cores. From statistical analysis of these results we conclude that half or more of the spheres >300 μm contain a single PGN, that less than half contain the previously described large FeNi metal cores and a smaller fraction contain no metal at all.

The analyses of 10 nuggets shown in Table 1 and Fig. 2 were obtained by energy dispersive X-ray analysis in an analytical scanning electron microscope (SEM). This approach was chosen in preference to the electron microprobe so that the beam could be precisely located on the nuggets. Pure element standards

were used and ZAF corrections were made to determine absolute concentrations. The L lines were used for elements heavier than Ni and peak integrals were determined by the 'super ML' multiple least squares fitting procedure⁷. The accuracy of this method for the moderately overlapping lines of Ru, Rh and Pd was confirmed by tests on synthetic spectra that closely matched those of the samples.

The analyses show that the nuggets are exotic alloys of group VIII refractory siderophile elements. More than 70% of the mass of a typical nugget is composed of Os, Ir, Pt and Ru. As seen in Fig. 2, five of the nuggets have unfractionated chondritic compositions for the platinum group elements. The mass of these elements in a nugget is consistent with formation by efficient concentration from a sphere of meteoritic metal similar in size to the host oxide sphere. Four of the nuggets have systematic depletions that correlate well with volatility; especially conspicuous are large depletions of Ni and Pd. One nugget is enriched in the more volatile platinum group elements.

A conceivable origin for the nuggets is that they are preserved relics from parent meteoroids and resemble platinum group nuggets found in carbonaceous chondrites^{8,9}. The nuggets reported here, however, are much more abundant and are generally larger than those of similar composition found in meteorites. The strongest argument against this source is that PGNs are never found in stony cosmic spheres. In our examination of polished sections of 1,000 stony (chondritic) cosmic spheres in the 300 μm to 1 mm size range, no PGN was observed.

The most likely origin of the PGNs is that they are the natural product of the heating and oxidation of small meteoritic metal particles during hypervelocity entry into the atmosphere. As the metal melts and begins to oxidize, elements more noble than iron remain in a rapidly shrinking metal bead surrounded by a growing shell of immiscible molten iron oxide (Fig. 3). If oxidation is not extreme, the sphere retains a relatively large Ni-enriched metal core. Nickel enrichment by this process is observed in deep-sea spheres¹⁰ and also in spheres associated with terrestrial impact craters^{11,12}. With more complete oxidation, most of the iron and nickel leaves the metal core and the result is a PGN. None of the platinum group elements is oxidized because of the low partial pressure of oxygen at the altitudes (70–90 km)¹³ where the spheres are molten. Oxidation of Os can occur at this pressure ($<10^{-5}$ atm for O_2) only if the temperature is below 600 °C (ref. 14); by the time the sphere is this cool, the nugget has been encased in solid iron oxide.

If heating is not severe (for example, due to low entry angle or velocity), the comparatively volatile elements like Rh, Ni and Pd are retained and a chondritic abundance pattern like the flat curves in Fig. 2 can be preserved. Extreme cases of heating result in vaporization of Ru, Pt, Rh, Pd and Ni and the product is a highly fractionated Os, Ir rich nugget, as seen in the lower

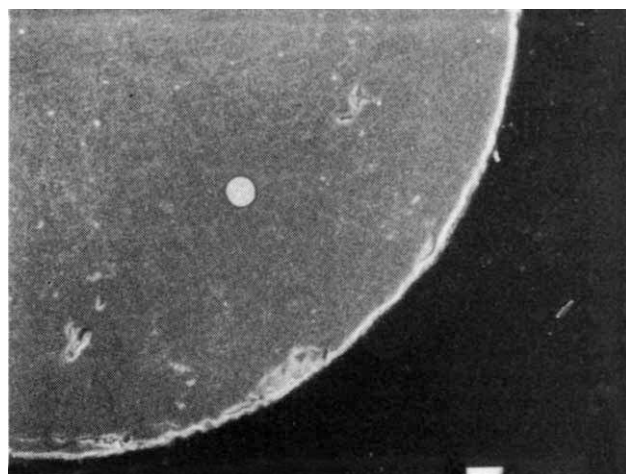


Fig. 1 Scanning electron micrograph of an 8- μm platinum group nugget in a polished section of an iron type cosmic deep-sea sphere. The host sphere is composed of magnetite and wustite.

Table 1 Analyses of 10 platinum group nuggets

	83H45	S114	8314	S-81	83H4	8318	8319	83A13	83A21	83110
Fe	5.7	6.8	6.9	5.6	6.1	6.9	9.9	7.0	4.3	5.6
Ni	9.2	13.3	10.6	2.8	2.7	17.7	56.1	25.3	4.0	1.9
Ru	21.4	19.5	23.0	16.9	19.7	17.2	7.5	12.9	19.7	15.0
Rh	3.9	5.0	6.3	0.9	—	4.0	2.0	3.4	1.4	—
Pd	—	1.2	5.1	—	—	0.5	5.8	3.9	—	—
Os	18.6	15.0	7.4	34.2	29.9	13.6	5.5	12.0	26.0	41.4
Ir	15.6	11.9	7.2	25.9	29.4	13.5	5.4	9.9	26.0	35.2
Pt	25.8	25.7	31.6	12.0	10.0	24.7	10.3	20.6	18.5	1.8
Total	100.2	98.4	98.1	98.3	97.8	98.1	102.5	95.0	99.9	100.6

curves of Fig. 2. Temperatures above 2,400 °C are required to produce significant Ru vaporization^{15,16} during the 5 s of peak heating that typical small particles experience near altitudes of 80 km (ref. 13). Significant heating above this temperature would result in vaporization of the oxide shell and the final product would be a bare nugget composed mostly of Os and Ir. Although these bare nuggets have not been observed, they may be more common than those embedded in oxide shells. The Pt/Ru/Rh-rich nugget (the highest curve in Fig. 2) cannot be produced by volatile loss from a source of chondritic composition. Unlike the other nuggets, this one must have originated from a source that has a Pt/Ir ratio higher than chondritic, possibly a III A,B iron meteorite¹⁷. One remarkable aspect of PGN formation in iron spheres is that the Pt group metals are efficiently concentrated by factors of $>10^5$ above chondritic on a time scale of a few seconds.

We conclude that typical half-millimetre particles of meteoritic metal are severely heated and oxidized during entry into the atmosphere and that these processes concentrate the refractory platinum metals into a small bead $\sim 10 \mu\text{m}$ in size. As described elsewhere¹⁸, there is evidence that most of the iron

cosmic spheres are actually not melted iron meteoroids but droplets produced by reduction in carbon-rich chondritic particles undergoing pulse heating at the top of the atmosphere. If this is the case, then a considerable fraction of the Os and Ir currently being accreted by the Earth could be in the form of PGNs. If this process occurs during major inputs of extraterrestrial material into the atmosphere, for example, at the end of the Cretaceous¹⁹, PGNs would form in enormous numbers. The nuggets should be quite stable to alteration and might survive as unique physical markers, while other debris weathers beyond recognition.

The formation of PGNs in iron cosmic spheres requires essentially total oxidation, a process that is only barely possible with the present abundance of oxygen in the atmosphere. Assuming that an impacting air molecule transfers all of its momentum to the decelerating meteoroid, then the particle will decelerate to 13% of its entry velocity after it has encountered an air column whose mass is twice the particle mass. Below this velocity the particle will not remain molten and PGN formation cannot occur. This simple momentum transfer calculation shows that during the time that a small iron meteoroid is strongly heated by atmospheric friction, it collides with a total number of oxygen atoms that is less than twice the number of iron atoms in the particle. Accordingly, we predict that iron cosmic spheres that formed before the abundance of oxygen in the atmosphere reached half its present level could not be totally oxidized during entry and should not contain PGNs. The study of PGNs in iron cosmic spheres recovered from dated stratigraphical layers may provide a means of determining when in the Earth's history the oxygen abundance rose to half its present level.

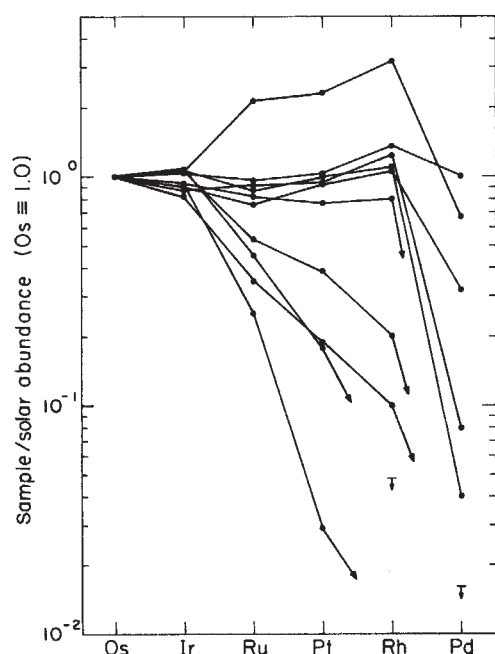


Fig. 2 Abundances of the 10 platinum group nuggets in Table 1 are normalized to the solar composition data of Anders and Ebihara²⁰. The data were not directly normalized to CI chondrites because Rh abundances have not been published for carbonaceous chondrites. The elements are plotted in order of increasing volatility from left to right. Temperatures required to produce vapour pressure of 1 torr range from 3,500 °C for Os to 1,900 °C for Pd. This vapour pressure produces vaporization at a rate of the order of $5 \times 10^{-3} \text{ cm s}^{-1}$ for a pure element^{19,20}. Arrows descending to the right indicate abundances below the indicated detection limits for Rh and Pd.

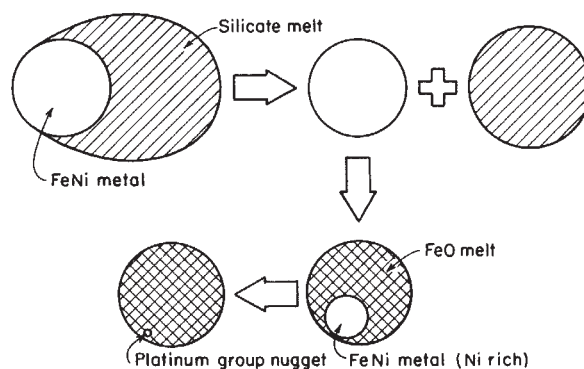


Fig. 3 The most common sequence of events that produces iron cosmic spheres containing platinum nuggets. An interplanetary dust particle melts during atmospheric entry and forms the composite spheroid of molten silicate and metal shown in the upper left. Due to their large density differences, the metal and silicate fractions separate by inertial forces forming a metal sphere and a silicate sphere¹⁸. The metal sphere develops a rapidly growing oxide shell and siderophile elements more 'noble' than iron concentrate into the shrinking metal core. When oxidation is nearly complete, most of the iron and nickel leave the core, resulting in a small nugget of nearly pure platinum group elements. Continued strong heating may vaporize the oxide shell, leaving only the nugget.

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Evidence from core-reflected shear waves for anisotropy in the Earth's mantle

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Seismologists have begun to recognize that the upper mantle at depth is anisotropic and that seismological data are not fully explicable if anisotropy is ignored^{1–5}. On entering an anisotropic zone a plane shear wave splits into two orthogonally polarized phases⁶; an effect observed by several researchers⁷, notably by Ando *et al.*^{5,8,9}. The near vertical ScS wave, an S wave reflected once at the core–mantle boundary, is one of the most suitable phases to study the phenomenon of shear wave birefringence because it is isolated from other phases and because its particle motion is nearly horizontal and thus free from S to P conversion. The split ScS shear waves travel with different velocities in the anisotropic zone and consequently there is a time delay between their arrival at stations. My aim was to determine this time delay and the azimuth of polarization using the ScS records of two horizontal components from the Japanese islands for a deep shock beneath the Sea of Okhotsk. From the total of 41 stations 34 show a polarization of the maximum velocity phase in the NNW–SSE direction with an average time advance of ~0.8s from the minimum velocity phase. The relevant anisotropic zone seems to be deep-seated.

The particle motion of the ScS wave before it enters an anisotropic zone must satisfy two conditions for the splitting phenomenon to be well observed. First, the particle motion should not be closely parallel to one of the principal axes of anisotropy as little shear wave splitting occurs in such cases. Second, from the focal mechanism point of view, the particle motion must have a large amplitude. The earthquake studied is a rare event that meets these two conditions at almost all the Japanese stations (see Table 1).

The hypocentral parameters of this earthquake are given by ISC as follows: date, 29 January 1971; origin time, 21 h 58 min 03.2 s; epicentre, 51.69° N, 150.97° E; depth, 535 km (pP–P); magnitude, $m_b = 6.0$. We used the data from the three-components seismographs with a natural pendulum period of ~5 s (courtesy of S. Mori of JMA) at 41 stations of the Japan

Meteorological Agency (JMA). The epicentre and the station distribution are shown in Fig. 1. The ScS rays are nearly vertical incident to each station because of their closeness to the epicentre. The original paper speed of the JMA seismograms was either 3 cm s⁻¹ (smoked paper) or 6 cm s⁻¹ (ink writing). All the ScS records of horizontal components were enlarged to an equivalent paper speed of 12 cm s⁻¹ and digitized every 0.2 s with an interval of 20 s. The digitized records were then corrected for the armlength of the pen recorder. Figure 2 shows several examples of the resultant horizontal traces; three methods were used to analyse them.

(1) Two horizontal records were synthesized into a particle motion diagram. In the absence of anisotropy the particle orbit was expected to be linear. After passage through an anisotropic zone, however, the particle motion would start with a movement along the polarization azimuth of the maximum-velocity phase and end with a movement along the azimuth of the minimum-velocity phase. The locus in between was expected to be approximately elliptical for a long-lasting signal. As seen in Fig. 2, the initial swing of the observed particle motion was generally directed towards the south-southeast. This swing was then followed by a counterclockwise elliptical rotation. It was difficult to identify the last swing of the ScS motion because of the coda waves. In some cases, however, a last swing towards the east-northeast was seen for example, at URA, ISN, KYO. The particle motion diagrams thus offer a diagnostic demonstration for the existence of anisotropy in the Earth with its maximum velocity polarized in the NNW–SSE direction.

(2) Two horizontal seismograms were rotated in a clockwise direction. An appropriate rotation would resolve the ScS wave

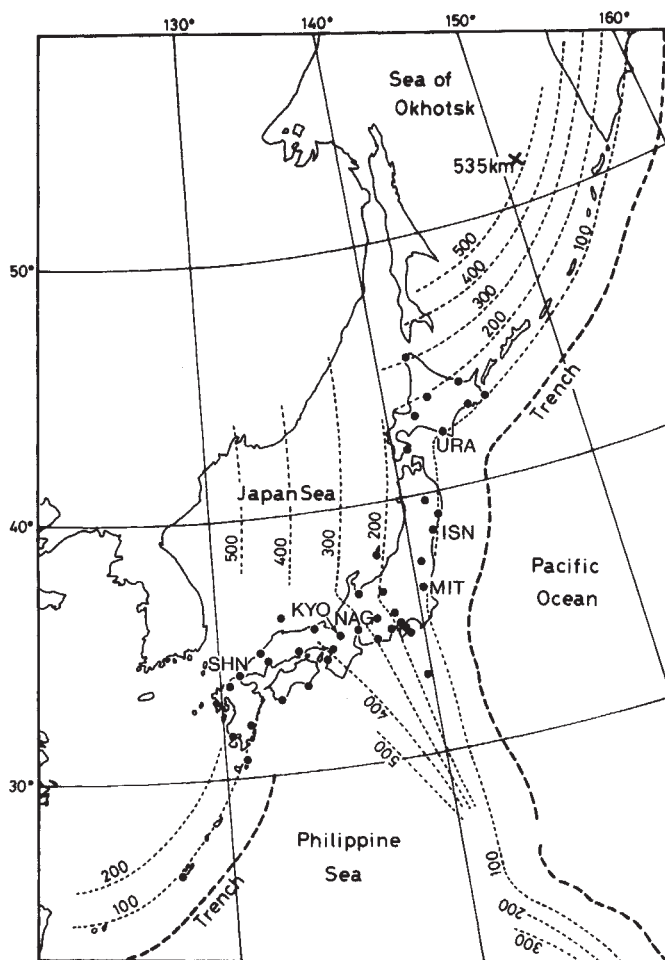


Fig. 1 Epicentre (x) and seismographic stations (●) used in this study. Letters indicate the six stations which appear in Fig. 2. Isodepth contours of deep earthquakes are also shown.

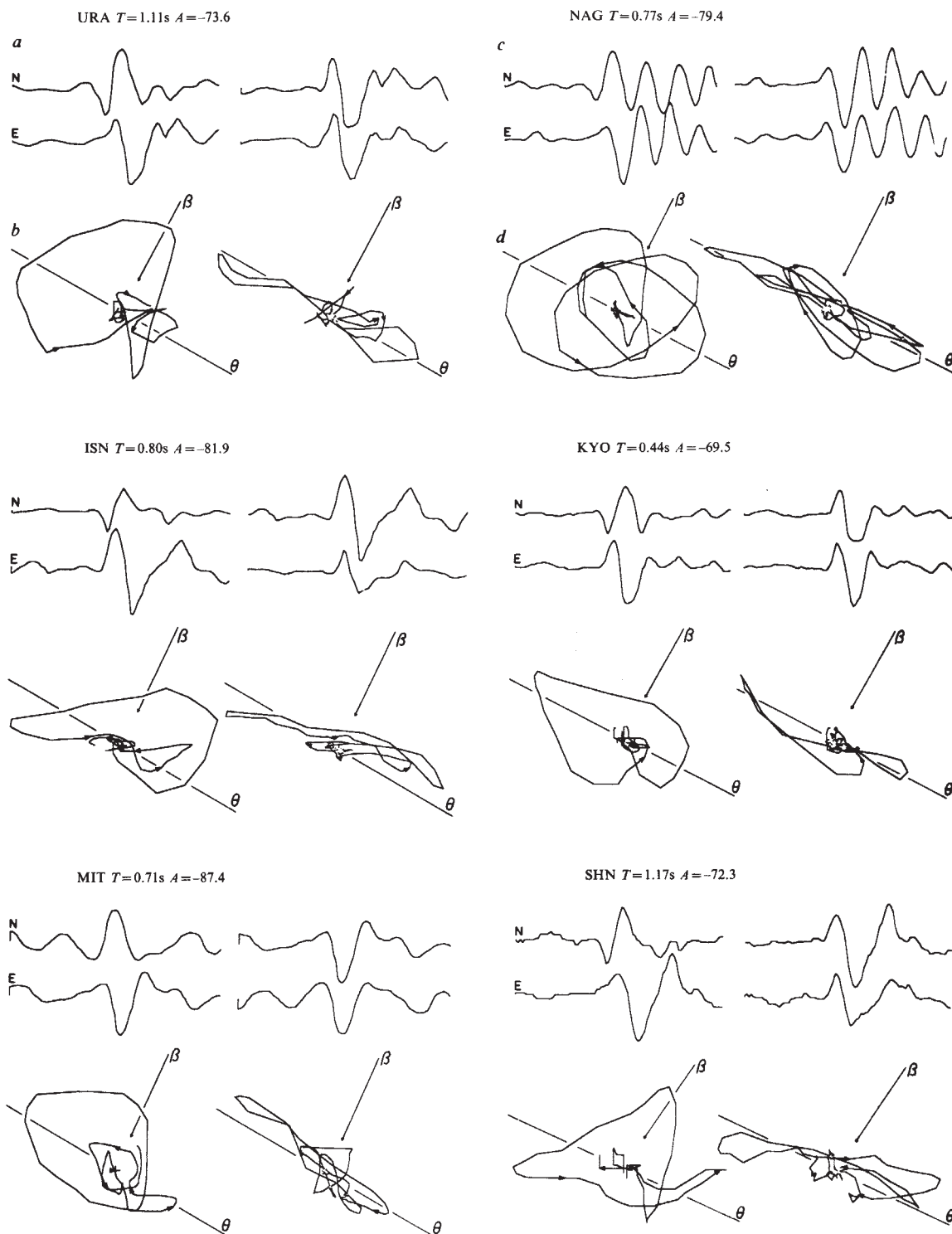


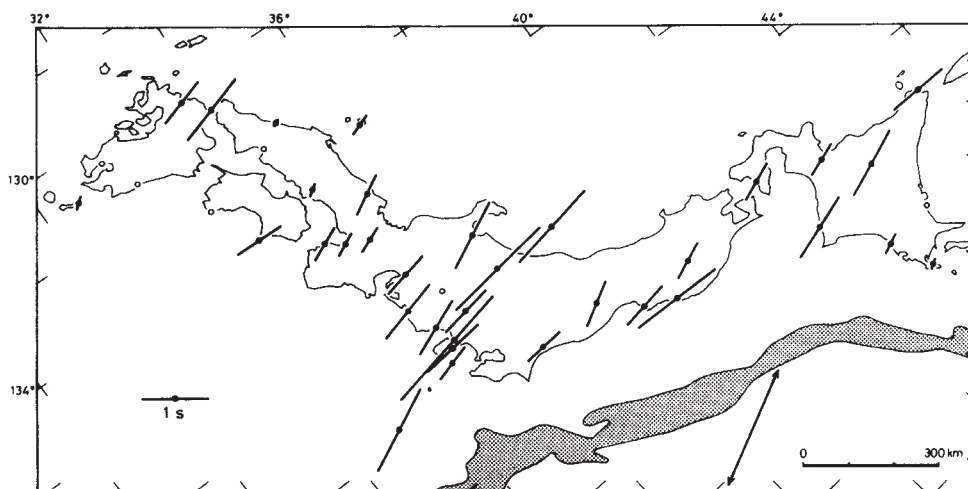
Fig. 2 ScS seismograms at six selected stations (see Fig. 1 for locations). The sampling time is 20 s from the beginning to the end of each trace. *a*, Original north-south and east-west seismograms. *b*, Original particle motion diagrams. β Indicates the incident azimuth of ScS wave and θ the direction of ScS motion expected from the focal mechanism¹⁰. The expected ScS motion is nearly transverse. *c*, Anisotropy-corrected seismograms obtained by a clockwise rotation A and a relative timeshift T . *d*, Anisotropy-corrected particle motion diagram.

into two orthogonally polarized phases whose wave-forms must be similar to each other. To find an appropriate azimuth of rotation a correlation was taken between the two rotated seismograms. Values for the rotation azimuth φ and the time lag τ yield the maximum correlation and were used to give the axes of anisotropy and a time delay between two polarized phases. (3) Two horizontal seismograms were rotated clockwise and then relatively shifted in time. Appropriate rotation and time shift would show a linear orbit in the resultant particle motion diagram. A straight line was fitted to the particle orbit of rotated

and time-shifted seismograms. The rotation azimuth φ and the time shift τ that give the best straight-line fit were, in general, consistent with those obtained by the second method, but did not coincide exactly. A set of φ and τ values which were in better agreement with the qualitative result from the first method were adopted as the final solution.

Figure 2 shows several examples of the seismograms rotated by an angle φ and relatively shifted by a time τ , and are thus corrected for anisotropy. Their waveform correlation is much better than that of the original north-south and east-west seis-

Fig. 3 Polarization azimuth of maximum-velocity phase of ScS wave and time delay between maximum- and minimum-velocity phases. Arrow indicates the azimuth of maximum velocity for horizontal propagation of P waves through the oceanic lithosphere¹.



mograms. Figure 2 also shows the particle motion diagrams corrected for anisotropy. Again the orbital linearity is much better than that of the uncorrected particle motion diagrams. As pointed out by Ando *et al.*^{8,9}, the linear trend of the corrected particle motion must agree with the direction of the ScS motion predicted by the P wave nodal plane solution. The direction of the ScS motion is calculated for the nodal plane solution given in Table 2 of ref. 10. The direction so calculated is attached to each of the particle motion diagrams corrected for anisotropy; the agreement is very good. The average of the azimuthal differences between the observed trend and the predicted one is $4 \pm 12^\circ$ (35 stations, see Table 1).

The result of the analysis is summarized in Table 1 and is shown in Fig. 3. The uniformity in polarization azimuth is remarkable. Out of 41 stations, 34 show a polarization of the maximum-velocity phase in the NNW–SSE direction. These stations are distributed over all the Japanese islands (Fig. 3). Only NAH, the most distant station from the epicentre, shows a polarization in the east–west direction. If this station is excluded, an average polarization azimuth of (N17°W–S17°E) $\pm 10^\circ$ is obtained for the maximum-velocity phase with

an average time advance of 0.8 ± 0.4 s from the minimum-velocity phase. A time difference of 0.8 s corresponds to a velocity difference of 4–5% in an anisotropic zone with a vertical extent of 100 km. The velocity difference would be reduced to a half if both the downgoing and upgoing ScS waves pass this anisotropic zone. For the remaining six stations I could not obtain the result with confidence for the reasons outlined in Table 1.

I conclude that there is an anisotropic zone in the ScS ray paths from the hypocentre to the Japanese islands. It is impossible to locate the site of anisotropy from the present analysis. It would, nevertheless, be rather surprising if the upper mantle above the deep seismic zone were to be so uniformly anisotropic over the whole Japanese islands in a variety of tectonic settings. In fact, Ando *et al.*^{8,9} observed that the anisotropic nature above the deep seismic zone in Central Honshu, Japan, varies considerably even on a horizontal scale of 100 km. The observed uniformity in polarization azimuth seems to indicate that the relevant anisotropy is deep-seated. For example, the anisotropy may reside in the descending slab of lithosphere. If this is the case, the ScS waves encounter the anisotropic zone at least once near

Table 1 Results of the earthquake analysis

Station	Δ (deg)	β (deg)	A_c (%)	θ_c (deg)	θ_0 (deg)	ϕ (deg)	τ (s)	Method	Station	Δ (deg)	β (deg)	A_c (%)	θ_c (deg)	θ_0 (deg)	ϕ (deg)	τ (s)	Method
WAK	8.8	41	81	117	146	180	1.0	R	OSH	18.9	23	88	120	129	165	0.6	R
ABJ	8.9	28	83	120	*	*	*	*	SHZ	19.0	25	87	119	104	159	1.0	R
NEM	9.1	22	84	121	107	152	0.2	C	NAG	19.3	27	86	118	119	169	0.8	C
KUS	9.8	25	84	120	110	158	0.3	C	HMM	19.5	25	87	118	103	167	1.1	R
ASA	9.8	33	83	118	95	160	1.1	R	TYK	19.9	31	85	116	120	150	0.6	R
SAP	10.8	34	83	118	133	162	0.6	R	SAI	19.9	34	84	115	112	159	0.4	C
URA	11.0	28	84	119	115	164	1.1	C	KYO	19.9	29	86	117	118	159	0.4	R
HAK	12.1	32	84	118	123	160	0.7	C	HIJ	20.3	20	89	121	111	157	1.4	R
MRK	13.8	26	85	119	121	158	0.6	C	OSA	20.3	28	86	117	109	156	0.4	R
OFU	14.2	24	86	120	125	185	1.5	R	WKY	20.9	28	86	117	118	157	0.6	R
ISN	14.9	24	86	120	106	172	0.8	R	OKA	21.0	31	85	116	103	147	0.2	C
FKS	15.8	25	86	119	104	151	0.7	C	HMD	21.6	33	84	114	110	153	0.1	C
AIK	16.3	29	85	118	104	170	1.5	R	HIR	21.9	32	85	115	*	*	*	*
MIT	17.0	23	87	120	130	177	0.7	R	MRT	22.1	28	86	116	110	182	0.8	R
NGN	17.6	27	86	116	109	174	1.8	R	SHN	22.9	33	84	114	102	162	1.2	R
TOY	17.9	29	86	117	96	154	1.1	R	ASZ	23.0	29	86	116	*	*	*	*
KOF	18.3	25	87	119	121	173	0.9	R	FKK	23.5	33	84	114	113	163	0.8	R
AJI	18.7	23	87	119	130	176	1.0	R	MYZ	24.4	30	86	115	†	†	†	†
MIS	18.7	24	87	119	112	170	1.8	C	KAG	25.1	31	85	115	†	†	†	†
IID	18.7	26	87	118	*	*	*	*	TAJ	25.6	29	86	115	93	140	0.2	C
									NAH	31.0	29	87	115	101	91	0.7	R

Δ , Epicentral distance. β , Station to epicentre azimuth (from north clockwise). A_c , Relative ScS amplitude expected from focal mechanism. θ_c , Direction of ScS motion expected from focal mechanism. θ_0 , Direction of ScS motion observed after anisotropy correction. ϕ , Polarization azimuth of maximum-velocity phase. τ , Time delay between two polarized phases. C, Correlation analysis of waveform. R, Regression analysis of particle motion.

* Particle motion difficult to interpret.

† Signal amplitudes of NS component too small.

the source and in most cases once again beneath Japanese stations (Fig. 1). The oceanic lithosphere seaward of the Kurile–Japan trench is strongly azimuth-dependent for horizontal propagation of P waves, the direction of maximum velocity being N25° W–S25° E¹. Note that this direction approximately coincides with the polarization azimuth of the maximum-velocity phase of near-vertical ScS wave. If the oceanic lithosphere consists primarily of olivine crystals whose *a*-axes are aligned in the NNW–SSE direction, both the P and S wave observations could be explained (see ref. 9). Of course, this argument is not valid, if the source of shear wave splitting were located below the depth of olivine–spinel transition.

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Subduction regression and oceanward migration of volcanism, North Island, New Zealand

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In the North Island of New Zealand, calc-alkaline igneous eruptions commenced in the early Miocene and continued into the Holocene in the central Taupo and Egmont areas, but no comprehensive synthesis exists to explain either the volcanic sequence or the regional structural setting. New data, reported here, from K–Ar ages and K₂O–SiO₂ indices for magma source depths indicate an oceanward migration pattern for volcanism, from north-west to south-east towards the Hikurangi Trench. Movement of this volcanic front throughout the Neogene and Quaternary was controlled by subduction zone regression¹ which followed rapid, shallow, non-volcanic Oligocene underplating of the island. In areas of accelerated subduction retreat, tensional tectonism caused widespread foundering of the Mesozoic greywacke basement.

Regional calc-alkaline volcanism in the North Island of New Zealand, of early Miocene–Holocene age, is confined to an area north-west of the main axial range of basement Mesozoic greywackes. Two geographically and petrologically distinct systems of eruptive rocks trend NW–SE parallel to the length of the island (Fig. 1) and contain contrasted calc-alkaline suites: basalts, andesites and rare dacites in the West Belt; and andesites, diorites, dacites, rhyolites and rare basalts in the East Belt^{2,3}. The two belts lie on opposite sides of the Stokes Magnetic Anomaly System⁴ which may represent the trace of an ancient convergence zone separating older, thicker (Palaeozoic) crust on the west from younger, thinner crust on the east. The NW–SE elongation of the volcanic belts has previously been regarded as an alignment of eruptive centres above a subduction system that flowed under the North Island from NE to SW, forming a Miocene–Pliocene interface between the Pacific and Indian plates^{5,6}. Subsequently, this inference has not been supported by the age and orientation of magnetic spreading lineaments in the South Fiji Basin⁷ or by the distribution of preliminary K–Ar dates within the volcanic belts⁸. Moreover, Quaternary calc-alkaline eruptive rocks are distributed along a line of volcanism which trends NE–SW from Taupo Volcanic Zone to Egmont

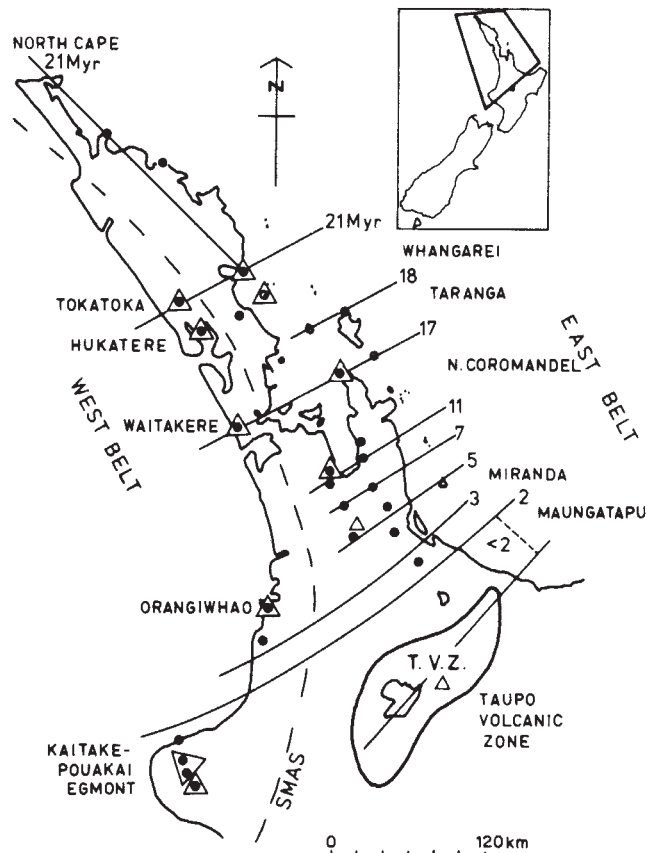


Fig. 1 The northern part of North Island, with the Stokes Magnetic Anomaly System (SMAS) separating the West Belt of calc-alkaline volcanism (Tokatoka to Egmont) from the East Belt (North Cape to Whangarei, and Whangarei to Taupo Volcanic Zone). ●, ○, including the boundary line for Taupo Volcanic Zone (TVZ), provide an indication of the distribution of calc-alkaline volcanic centres in North Island and also give specific locations for K–Ar ages (total of 93 dates) from which the time-lines were determined. △, with place names, are localities for K₂O–SiO₂ estimates of magma source depths shown in Fig. 2.

(Fig. 1) and is almost orthogonal to the East and West Belts; the related modern subduction system, dipping to the north-west, is well defined from seismic data and it originates at the Hikurangi Trench where the Pacific plate descends at an initial shallow angle (~15°) below the edge of the (North Island) Indian plate and then steepens to 50–60° below Taupo–Egmont^{6,9}.

Additional K–Ar ages for andesites, diorites and dacites within both volcanic belts have provided time-lines (Fig. 1) which represent the oldest dated rocks, and thus the initiation of volcanism, at eruptive centres throughout the northern North Island, and two orientations are apparent.

(1) In the northernmost part of the island, a NW–SE 21-Myr time-line lies along the eastern side from North Cape to Whangarei. Andesites in this zone are unique within the North Island by containing garnet or high-pressure igneous mineralogies, and by having a close association in the field with ophiolite massifs; their origin is believed to belong to a brief magmatic event which accompanied ophiolite obduction at ~23–25 Myr, at the end of Oligocene seafloor spreading in the South Fiji Basin^{7,10}.

(2) Time-lines with NE–SW orientation record southeastward migration of calc-alkaline volcanism over a distance of 320 km from Whangarei (East Belt)–Tokatoka (West Belt), with oldest ages of 21 Myr, to the Quaternary volcanoes of the Taupo Volcanic Zone (East Belt) and the Egmont group (West Belt). The parallelism of the time-lines and their progressive younging from north-west to south-east are evidence for continued migra-

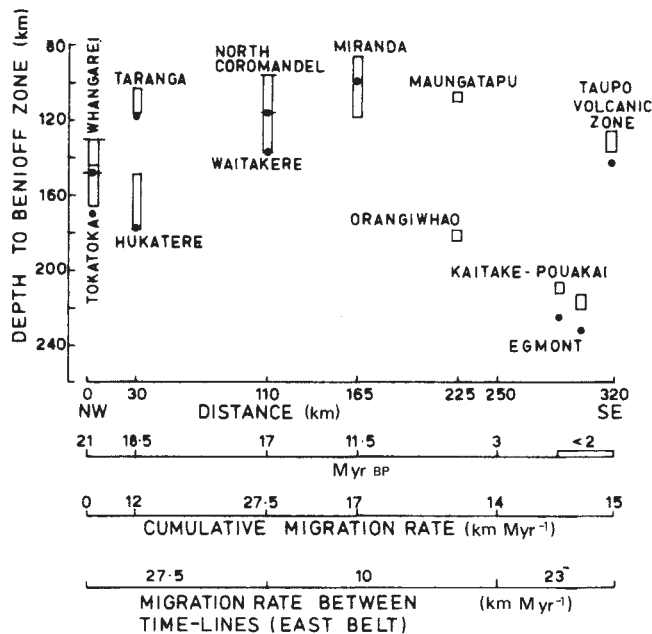


Fig. 2 K_2O-SiO_2 estimates of depth to magma sources, plotted against NW-SE distance from Whangarei-Tokatoka (21 Myr time-line) to Taupo Volcanic Zone-Egmont (<2 Myr) as measured along the East Belt. Rectangles show the total estimated depth range at the centre of the Benioff zone based on the K_{55} and K_{60} method¹¹; ●, the maximum estimated depth to the inferred base of the Benioff zone using the $K_{57.5}$ method¹². The depth shown for Maungatapu is a minimum (using only K_{55} data) and for Orangiwhao is a maximum (using only K_{60} data).

tion of the subduction profile which currently underlies the Taupo-Egmont volcanic centres, with a north-west polarity. Thus, from early Miocene to present day, a mobile volcanic front has accompanied the southeastward retreat of a magma-generating subduction surface (Benioff zone) which developed as a result of oceanward regression of a precursor shallow underplating system that originally must have extended north-west from the Hikurangi Trench for about 650 km inland to the Whangarei-Tokatoka line.

By the potash-silica method, estimates of depths^{11,12} for generation of the andesites (Fig. 2) indicate that East Belt lavas consistently originated at shallower levels than those of the West Belt where the greater depth for magma sites, all lying to the west of the Stokes Magnetic Anomaly System, may be related

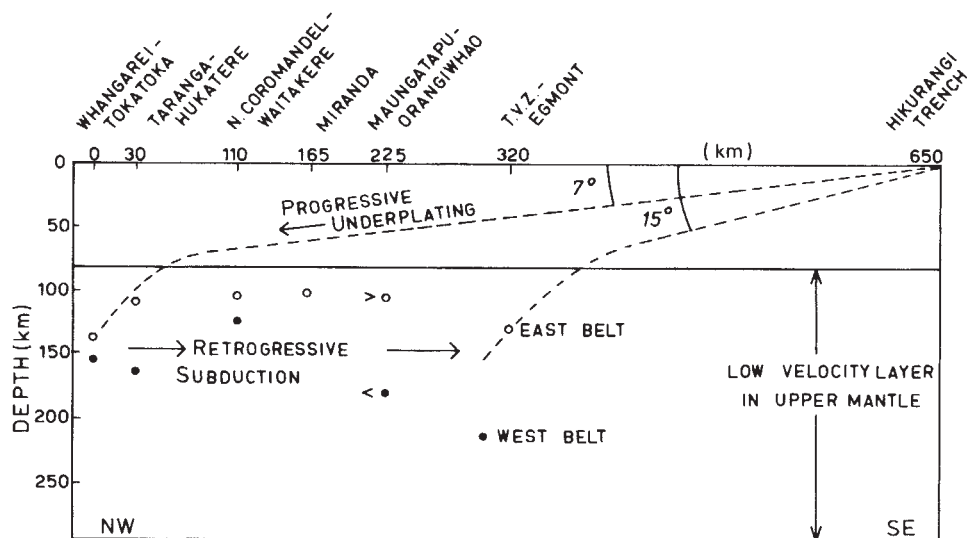
to the presence of a thicker, older sialic basement. Estimation of magma source levels from K_2O-SiO_2 indices is validated particularly for Quaternary lavas of the Taupo Volcanic Zone (124–142 km) and the Egmont volcanoes (206–232 km) where calculated depths lie within the seismically defined profile of the underlying modern Benioff zone¹³. For calc-alkaline suites in the East Belt, shallow origins are indicated also by the general rarity of basalts and by active crustal contamination of magma sources in producing extensive rhyolites and regional hydrothermal metalliferous alteration^{14,15}. When compared with this evidence, $^{87}Sr/^{86}Sr$ initial ratios^{8,15} seem to be anomalous^{12,16}, with higher values in the apparently shallower-sourced East Belt (basalts = 0.7041–0.7044; andesites rarely = 0.7035–0.7045, commonly = 0.7050–0.7065) than in the West Belt (basalts = 0.7030–0.7038; andesites = 0.7040–0.7055). The main anomaly is not the isotope ratios, but rather that mantle-derived magmas have failed to react appreciably with the Palaeozoic, more radiogenic crust of the West Belt.

The time-lines for 3 and 2 Myr develop a more westerly trend where they cross the West Belt because of the combined effect of greater depths for those magma sources and the configuration of the regression path for the subduction zone (Fig. 3); these youngest time-lines thus make an angle of $\sim 20^\circ$ with the clearly defined regional alignment of Holocene volcanic centres in the Taupo Volcanic Zone (East Belt). In view of this geometrical relationship, the older time-lines (21–5 Myr) similarly could have been divergent to the strike of the subduction surface which, at all stages of retreat, possibly approximated the trend of the Taupo Volcanic Zone.

Southeastward movement of the volcanic front, as measured along the East Belt by time-lines 21, 17, 3 and <2 Myr, shows that the rate of migration was not regular (Fig. 2). Initially, at the commencement of subduction regression, migration between time-lines 21 and 17 Myr proceeded at a rate of 27.5 km Myr^{-1} and the intervening region was subjected to pronounced tensional tectonism that produced widespread foundering of the greywacke basement (Fig. 4) and a deep basin for concurrent early Miocene flysch sedimentation¹⁷. In contrast, the following period of relatively slow migration at 10 km Myr^{-1} , between time-lines 17 and 3 Myr, traversed a region where the greywacke basement was extensively blockfaulted but not notably depressed, so that it persisted as a shallow marine shelf for calcareous Miocene-Pliocene sediments. Subsequent acceleration in the migration rate, at 23 km Myr^{-1} for the interval 3–<2 Myr, was again characterized by tensional collapse of the greywacke basement to depths of 5 km and accompanied by voluminous eruptions of silicic calc-alkaline volcanics in the Taupo Volcanic Zone^{9,18}.

Underplating of the North Island by the Pacific plate, before the early miocene, would have involved rapid shallow

Fig. 3 SE-NW profile along the East Belt from Hikurangi Trench to Whangarei-Tokatoka (21 Myr time-line) with averaged $K_{55}-K_{60}$ estimates of magma source depths for East Belt (○) and West Belt (●). Progressive shallow underplating during the Oligocene was followed by Miocene-Holocene subduction retrogression to the profile of the present Benioff zone¹³ beneath Taupo Volcanic Zone and Egmont.



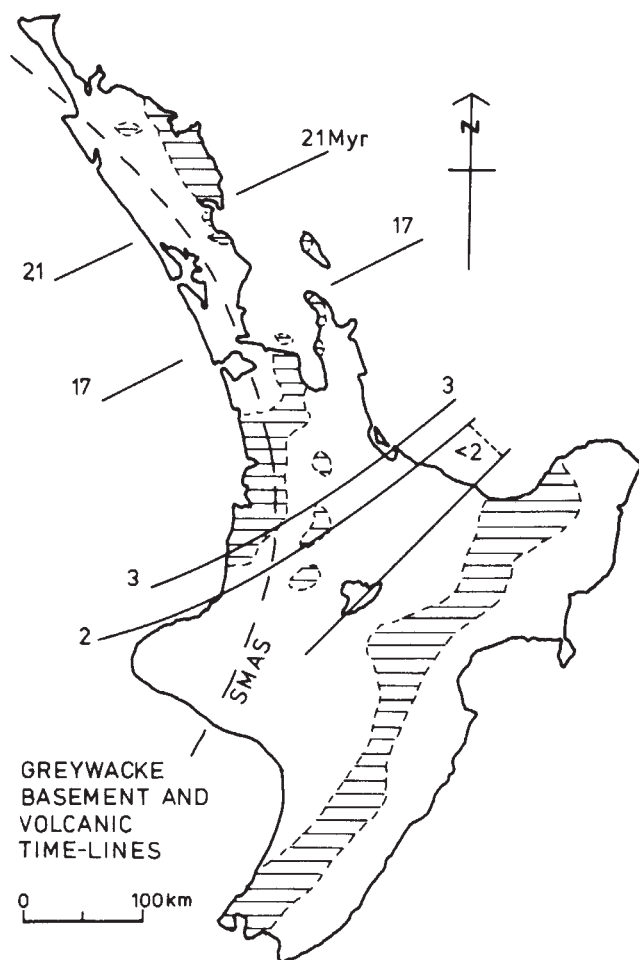


Fig. 4 North Island Mesozoic greywacke basement outcrop pattern, developed by tensional tectonic collapse within regions of accelerated subduction regression between volcanic time-lines 21–17 Myr and 3–<2 Myr.

subduction not accompanied by volcanic activity for a distance of 650 km from the Hikurangi Trench to the Whangarei–Tokatoka line, a tectonic style which is similar to the amagmatic Laramide event in the western Cordillera of North America¹⁹. The geometry of the underplating process can be achieved by assuming a low initial trajectory of 7° (Fig. 3) which implies a rapid rate of convergence between the upper plate and the subducting slab, and a buoyant strong mechanical coupling between the two plates^{20,21} which is supported by the presence of an accretionary prism of scraped-off Oligocene–Quaternary marine sediments on the landward side of the shallow Hikurangi Trench (Fig. 5). Marginal basin spreading during the Oligocene (35.5–25.5 Myr) caused offset of the North Island towards the south-east, relative to the South Fiji Basin⁷, in opposition to the general westerly direction of motion at that time for the Pacific plate²², thus enhancing the velocity of convergence at the subduction interface. This 10-Myr period of accelerated convergence, with the North Island (Indian) plate overriding the Pacific plate, could have provided the mechanism necessary for Oligocene underplating at a rate of at least 65 km Myr⁻¹. The end of seafloor spreading in the South Fiji Basin (25.5 Myr)⁷ was followed by southwestward obduction of ophiolites onto the North Island (25–23 Myr)¹⁰ and by a reduction in convergence velocity for the shallow subducting slab which, with a consequent increase in gravitational sinking rate and a steepening profile^{23,24}, reversed its mode at 21 Myr from north-west directed progressive underplating to southeastward subduction

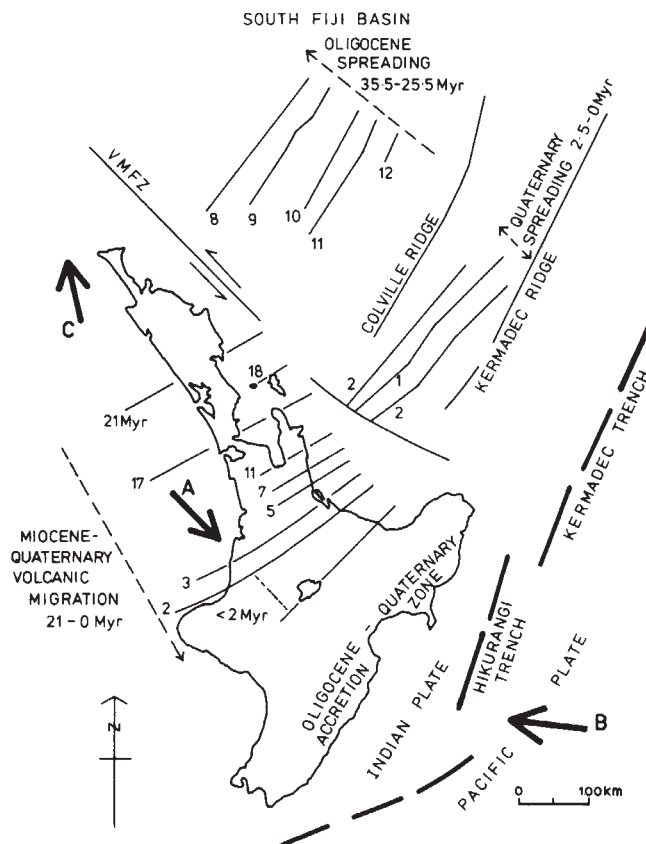


Fig. 5 Late Tertiary and Quaternary lineaments in the North Island and adjacent oceanic area. Oligocene marginal basin spreading (magnetic anomalies 12–8) produced left-lateral displacement along the Vening Meinesz Fracture Zone (VMFZ), and after Neogene quiescence was followed by Quaternary spreading (anomalies 2–1) between the Kermadec and Colville Ridges⁷. Arrow A shows Oligocene motion of North Island (Indian plate) relative to the South Fiji Basin. Arrow B is indicative of various directions within the west quadrant for absolute motion of the Pacific plate from Oligocene (anomaly 13) to Holocene, and arrow C is the present absolute motion vector for the North Island^{22,25,26}.

regression towards the Hikurangi Trench. The patterns for seismic activity¹³ and younging of volcanic ages across the modern Benioff zone (Figs 1, 3) suggest that subduction retreat towards the south-east still continues.

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A late Devensian ice-free area and possible interglacial site on the Isle of Lewis, Scotland

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At the time of the last glacial maximum the last Scottish ice sheet has been considered by some^{1,2} to have extended to the continental shelf edge (Fig. 1a) whilst others^{3,4} have suggested that there were ice-free areas in northern Scotland. No radiocarbon-dated stratigraphical evidence has been published to support either interpretation. We present here data from the Isle of Lewis establishing that the shelly deposits from the north of that island are of late Devensian age but which also show that a late Devensian ice margin lay across northern Lewis leaving part of the island ice-free. A peat, possibly of interglacial status, has been found in the ice-free area. The last Scottish ice sheet therefore did not pass beyond the Outer Hebrides and may not even have reached the north of the island chain. This evidence is also of interest to the debate on the relative expansions of the northern and southern margins of the Laurentide and Scandinavian ice sheets at the last glacial maximum^{5,6}, for it suggests a restricted glacial expansion of the northern margin of the last British ice sheet at the time of its greatest southerly extent.

Detailed mapping at a scale of 1:10,560 and recording of all natural sections in the north of Lewis have confirmed McCann's⁷ observations that on part of the north-west Lewis coast, a raised beach occurs at an altitude of ~12 m (local OD)⁸. Along the stretch of coast marked a-a' in Fig. 1b the beach is *in situ* and up to 150 m broad. Frequent cryoturbation structures at the top of the gravels together with solifluction and head deposits that rest on and partly incorporate the beach gravels establish that the beach predates at least one period of periglaciation^{4,9}. The beach is best developed along the coast south of Galson and is here termed the Galson Beach. Although composed dominantly of material derived from the local Lewisian gneisses, the beach also contains frequent red and purple sandstone clasts that diminish in abundance southwards. The present study has also confirmed that along section a-a' of the coast the beach occasionally overlies a till which rests on a wide (up to 150 m) rock platform^{4,7,9} at an altitude of ~9 m (local OD)⁸.

South-west of Melbost Borve (a, Fig. 1b) Peacock⁴ noted that the gravels of the Galson Beach were overlain by, or incorporated in, a till and he suggested that the late Devensian limit of Outer Hebridean ice occurred in this vicinity. North-east of South Dell (a', Fig. 1b) the complex shelly glacial drifts can also be observed to overlie Galson Beach deposits. At Bad an Fhithich (BF, Fig. 1b) beach gravels (which may be an erratic) have been reported^{4,7} to overlie till although at the same locality stratified beach cobbles and sands resting *in situ* on bedrock are truncated by and overlain by till.

Detailed mapping of the shelly glacial deposits indicates that only one phase of glacial activity is represented. Complex glaci-tectonic structures and large erratics of marine clays and gravels have given rise to Baden-Powell's¹⁰ interpretation of two glacial phases with an intervening period of interglacial marine deposition. These glacial deposits terminate abruptly on their southern margin and this limit can be traced across north Lewis to a major drift ridge up to 30 m high that extends for over 6 km to the south-southeast to reach an altitude in excess of 110 m (Fig. 1b). North and east of the ridge are extensive glacial deposits, areas of dead-ice terrain and a sequence of meltwater channels trending north-northwest.

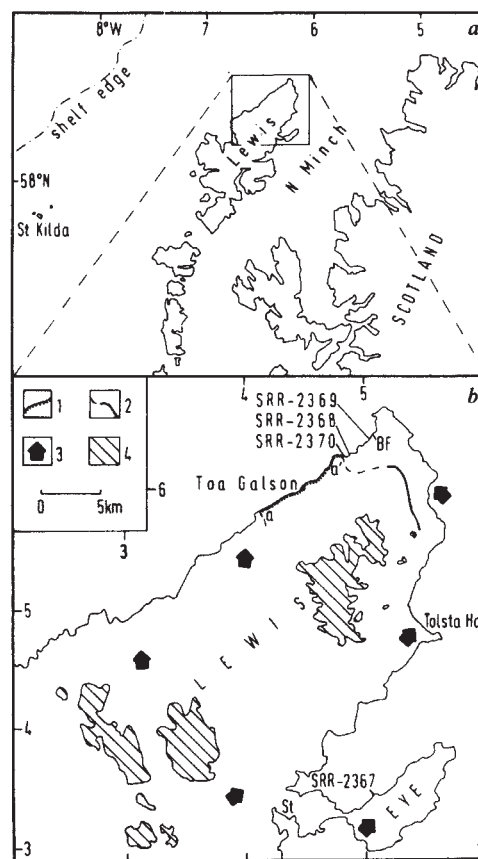


Fig. 1 a, Location of Lewis and b, Northern Lewis. 1, Stretch of coast (a-a') where the Galson Beach is *in situ* and not overlain by glacial deposits; 2, ice margins, continuous line representing the end moraine in north Lewis; 3, generalized directions of last ice movement; 4, land over 122 m (400 ft).

The drift ridge is interpreted as an end moraine of the last ice mass to occupy the north of Lewis and the trend of the moraine, together with the direction of slope of the meltwater channels indicates that the ice had a surface gradient from south-southeast to north-northwest. Abundant purple and red sandstone clasts in the glacial deposits are most probably derived from Mesozoic¹¹ sedimentary rocks that floor the North Minch close offshore and they cannot be taken as evidence for the presence of Scottish mainland ice which their original attribution to the Torridonian was thought to imply¹². Indeed, the last ice in this area may have been no more than Outer Hebridean ice recurving round the upland of northern Lewis on to the low ground farther north.

Samples of shell debris from three localities in the middle or base of these glacial deposits (Fig. 1b) have been radiocarbon dated (Table 1) and all gave middle Devensian ages. The shell faunas in the glacial deposits are derived from a variety of oceanographic provinces and thus very probably represent a range of ages from the ice-free period before the last ice advance. Hence the 34,000–39,000 yr BP sample ages are likely to result from a mixture of late Devensian and middle Devensian (or older) shells. Amino acid analyses of ice-transported shells from north Lewis suggest a late Devensian age (D. Q. Bowen, personal communication), supporting this interpretation. The evidence therefore indicates that the last glaciation in the north of Lewis was during the late Devensian. Expansion of the Outer Hebridean ice cap during the late Devensian is also indicated by the date on the ice-transported shells from Garrabost on the Eye Peninsula (Fig. 1b, Table 1), the last ice movement there as determined by carry of local erratics being from south-west to north-east.

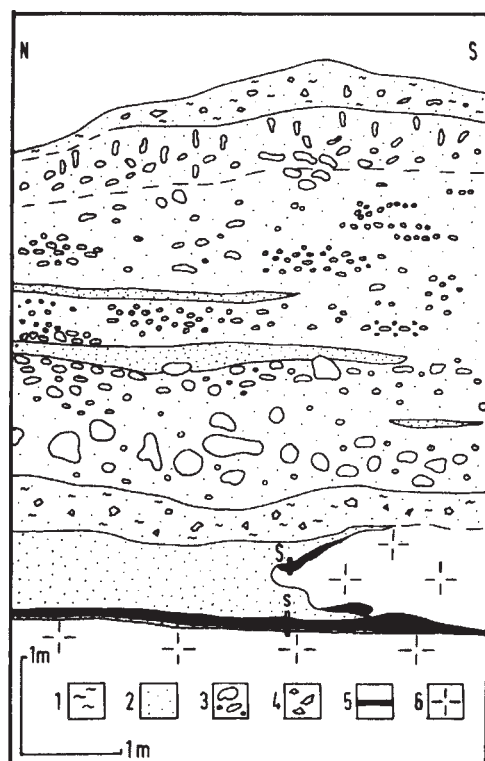


Fig. 2 Section south of Toa Galson. 1, Silt and clay; 2, sand; 3, rounded boulders and cobbles; 4, angular pebbles and gravel; 5, peat; 6, weathered or decomposed bedrock. Note cryoturbated upper ~75 cm of beach gravels. Samples for pollen analysis taken from locations marked S.

The establishment of the latest glacial event in the north of Lewis as being late Devensian in age and the stratigraphical evidence that the Galson Beach underlies or is incorporated within these glacial deposits implies that the stretch of coastline a-a' (Fig. 1b) was ice-free during the late Devensian. A wave-eroded section at the front edge of the raised rock platform ~100 m south of Toa Galson (Fig. 1b) is particularly significant in this context, because there a peat that postdates the formation of the raised rock platform and the glaciation of this part of the coast, but predates the deposition of the Galson Beach, has been found. The stratigraphy is shown in Fig. 2.

The peat has been distorted and partly eroded by a slump of decomposed bedrock that is contemporaneous with the overlying medium-coarse iron-stained sands. Here we term the main peat body as the lower peat, Site A, the uppermost disturbed peat lens is the upper peat, Site A and a peat lens that occurs several metres farther south in the section is Site B. Between the iron-stained sand and the beach gravels is a layer of angular locally-derived clasts in a silty-sand matrix that shows minor silt lenses and weak lamination that are typical of solifluction.

Samples for pollen analysis were removed from the peat and adjacent sediments (Fig. 2) and the spectra recorded in the three peat lenses are shown in Fig. 3. In the main peat body (lower peat A) the lower samples are dominated by Gramineae and Cyperaceae but Cruciferae, *Plantago* spp., *Rumex* and *Saxifraga* are consistently represented. An open, floristically-diverse grassland is suggested with wetter areas providing a habitat for the sedges and certain species, possibly, of the Umbelliferae. With continued accumulation of the peat there is a steady increase in Ericaceae (including *Empetrum*) pollen which suggests a progressive change in the local vegetation and in the upper part of the peat the pollen spectra imply an acid heath vegetation interspersed with grassland communities. *Empetrum nigrum* is generally considered to be an oceanic species^{14,15} and the change in the pollen spectra may reflect increasingly oceanic conditions with soils becoming leached and base-poor. Throughout the

Table 1 Radiocarbon dates on ice-transported marine shells from Lewis

Site	Laboratory no.	$\delta^{13}\text{C}$ (‰)	^{14}C age (yr BP)
West Dell Sands	SRR-2368	(o) +2.3	37,070 ⁺⁹⁷⁰ -860
		(i) +2.3	34,470 ⁺⁷²⁰ -660
			+1270
Traigh Chumil	SRR-2369	(o) +1.2	39,850 ⁺¹¹⁰⁰ -1100
		(i) +1.9	39,500 ⁺¹²⁷⁰ -1100
			+1130
Peicir	SRR-2370	(o) +1.6	37,320 ⁺⁹⁹⁰ -990
		(i) +1.4	35,630 ⁺¹⁵²⁰ -1270
			+1130
Garrabost	SRR-2367	(o) +1.6	26,300 $\pm$ 320
		(i) -3.0	23,000 $\pm$ 230

Pretreatment for all these samples consisted of discarding 25% by weight of the bulk sample by acid hydrolysis with the remaining carbonate being divided into equally sized 'outer' (o) and 'inner' (i) fractions by further acid treatment.

Table 2 Radiocarbon dates on buried peat layer at Toa Galson

Fraction	$\delta^{13}\text{C}$ (‰)	d^{14}C (‰)	^{14}C age (yr BP)
(a)	-29.3	-1000.9 $\pm$ 0.7	>47,150
(b)	-29.2	-1000.8 $\pm$ 0.8	>46,640
(c)	-29.7	-1001.1 $\pm$ 1.9	>39,100

Fraction (a) was composed of $\geq 125 \mu\text{m}$ material that was acid washed in 2M HCl; fraction (b) consisted of $< 125 \mu\text{m}$ material that was soluble in 0.5 KOH; and fraction (c) was the residual $< 125 \mu\text{m}$ material following the alkali treatment. Fraction (b) should relate to humic acid contamination and fraction (a) should contain the coarse parts of any modern rootlets that may have penetrated the peat. The acid wash was to remove any carbonates that may have been precipitated in the peat.

pollen profile there are low percentages of arboreal pollen, principally *Pinus*, although *Picea*, *Betula*, *Alnus* and *Abies* grains were also recorded. These are considered to have been derived by long-distance transfer and, with the possible exception of small areas of *Salix* and *Juniperus* scrub, the environment is interpreted as being essentially treeless.

Plant macrofossils were poorly preserved in the peat, leaf fragments of *Sphagnum imbricatum* being found in the lower part and *Viola* seeds occur throughout (P. Hulme, personal communication). Undifferentiated *Viola* seeds have a wide range of occurrence during the Pleistocene¹⁶, but *S. Imbricatum* is of greater interest. Dickson¹⁷ indicates that in pre-Lateglacial times *S. Imbricatum* has only been recovered from interglacial deposits with the exception of one possibly-derived attribution to the Anglian.

The upper surface of lower peat A is clearly erosional but the basal sample of the overlying sand was found to be polleniferous. This revealed a pollen spectrum very similar to the base of lower peat A. As some, if not all, of the pollen grains in this sample may have been derived from erosion of the peat no environmental change may be interpreted from this single sample. The pollen spectra from upper peat A and from site B (Fig. 3) are very similar to those from the upper part of lower peat A with a strong Ericaceae (and *Empetrum*) component. These analyses confirm that the peat lens was originally more extensive and has been eroded and disturbed by the subsequent events at the site.

A sample was removed for radiocarbon dating from the upper part of lower peat A. The sample was split into three fractions

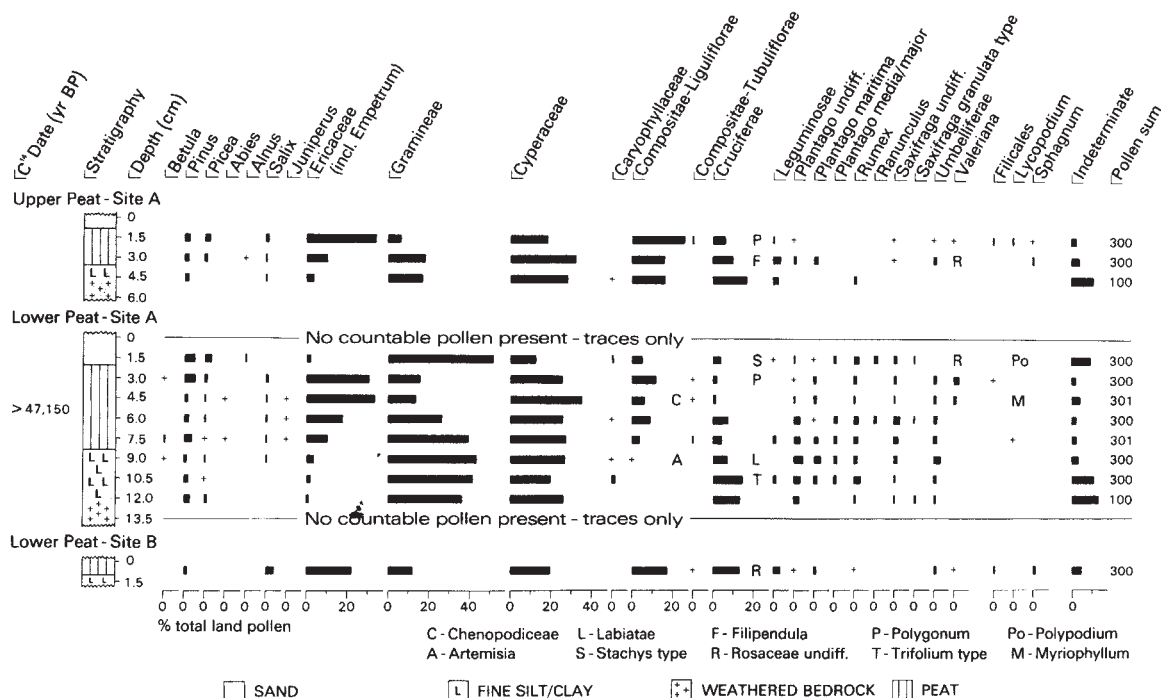


Fig. 3 Percentage pollen diagram from Toa Galson. Samples for pollen analysis were prepared using conventional procedures¹³, except that residues were passed through microsieves (aperture 10 μ m) to concentrate pollens and spores. Pollen preservation was good in the peat layers but in the minerogenic sediments large numbers of corroded and amorphous grains were found.

following a procedure designed to isolate the possible contaminants (carbonates, organic acids, modern rootlets). Details of the pretreatment and the radiocarbon results are given in Table 2. The agreement between all fractions indicates minimal contamination and a sample age of >47,150 yr BP.

The essentially treeless environment that has typified the northern and western islands of Scotland during the Flandrian and presumably previous interglacials and interstadials poses considerable problems in assessing the status and correlation of pre-late Devensian pollen-bearing sediments. Two other such sites are known from the Outer Hebrides, Tolsta Head^{18,19} and St Kilda²⁰ (Fig. 1). As both these sites are associated with finite radiocarbon ages and have significant differences in their pollen spectra from that of Toa Galson, the sites are not thought to be the same age. Farther afield, however, there are certain similarities between the Toa Galson pollen profile and that from Sel Ayre, Shetland²¹ where a vegetational development from grass and fern dominated communities to dwarf-shrub heaths dominated by Ericaceous shrubs, including *Empetrum*, was recorded. The local environment was treeless and the Sel Ayre site was assigned an interglacial age on the basis of inblown tree pollen and tentatively ascribed to the Ipswichian. The progressive changes of vegetation at Sel Ayre were suggested to represent an interglacial cycle in a treeless environment and if this model is correct then the Toa Galson peat may tentatively be assigned an interglacial status. Such an interpretation is in agreement with the occurrence of macrofossils of *Sphagnum imbricatum*, the floristic diversity of the grassland communities, the occasional thermophilous inblown tree species and the radiocarbon age. There is, however, no evidence that allows the site to be ascribed to a particular interglacial.

Models of the last ice sheet showing Scottish ice extending to the continental shelf edge depict the north of Lewis as being covered by 1,000–1,500 m of ice^{1,2} but our evidence demonstrates that part of this area was actually ice-free. This implies a relatively restricted late Devensian glaciation along the north-west margin of the Scottish ice sheet, the ice perhaps extending no more than 100 km from the major northern Scottish mainland ice shed. Yet at this time the ice sheet reached the Midlands of

England, south-west Wales and southern Ireland. In recent years there has been considerable discussion as to the relative expansions of the northern and southern edges of the Laurentide and Scandinavian ice sheets at the last glacial maximum^{5,6}. In contrast to the traditional view of synchronous glacial expansion, a 'minimalist' school has suggested that the northern margins had a relatively restricted glaciation at the time of maximum southerly advance. We suggest that a similar north-south contrast typified the late Devensian British ice sheet and palaeoclimatic models that seek to explain the north-south contrasts of the larger ice sheets must also be capable of explaining a marked climatic gradient across the more southerly British ice sheet.

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Interpretations of the magnetostratigraphy of the Hadar hominid site, Ethiopia

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As part of an investigation to determine the age of the hominid-bearing strata of the Hadar Formation of Ethiopia we have determined the magnetostratigraphy of two separate sections of the strata. Two major zones of reversed polarity occur within the predominantly normally magnetized sequence, one at the top and the other in the middle. There are difficulties with the absolute age determinations of volcanic units within the strata which result in uncertainties in correlating the Hadar magnetostratigraphy with the standard magnetic reversal time scale. The age of the upper part of the section is established by the BKT-2 tuff at ~2.8 Myr (refs 1–4). The K–Ar dating of the Kadada Moumou basalt which lies in the middle of the sequence yields variable results ranging from 3.1 to 3.6 Myr (refs 1–4). Regional geochemical⁵ and biostratigraphical⁶ studies suggest the younger age for the Kadada Moumou basalt. We show here that this is also the age that results in the most consistent interpretation of the observed magnetostratigraphy of the formation, which is that the Hadar Formation was deposited during the Gauss Normal epoch and that the two reversals represent the Kaena and Mammoth events within that epoch.

The Hadar Formation is exposed in a badlands topography along the Awash River valley to the south-west of the town of Mille (Fig. 1a). As the beds of the Hadar Formation are horizontal or dip gently and only minor faulting is present, continuous stratigraphical sections can be sampled along the main wadis. Two major sections were sampled (Fig. 1b) along the Kada Hadar drainage (called the camp section) and along the Ounda Hadar drainage (called the Ounda Hadar section).

Details of sampling and laboratory procedure are given elsewhere^{2,7}. Samples in the camp section were spaced at 0.5-m stratigraphical intervals, the Ounda Hadar section was sampled at more widely spaced intervals, the lower part with a large and variable interval of up to 3 m and the upper part at a 1-m interval. The natural remanent magnetization (NRM) varied, in the range of 1–50 mA m⁻¹. All samples were progressively demagnetized in alternating fields. The NRM directions of magnetization were variable; normal and reversed directions were found as well as an appreciable number of intermediate directions; the intermediate directions gradually disappeared during the progressive demagnetization. The directions of magnetization of some samples did not stabilize until high fields were applied (50–75 mT, Fig. 2b).

The cleaned magnetic declination of the camp section samples are shown in Fig. 2a in stratigraphical order with the previously

published upper part of the Ounda Hadar section. The data for the camp section differ from previously presented data^{2,7} in that alternating field demagnetization was continued to higher fields. The higher field demagnetization better defines the reversed intervals (see Fig. 2b). The comparison of the two sections is very favourable in that the fine scale of variation in declination between the two sections matches well. Although there are normal directions of magnetization within both reversals which are quite similar in both sections, their origin is not certain.

There are few radiometric data to tie the observed polarity sequence to the standard magnetostratigraphical scale because there are only two horizons in the Hadar Formation which can be used to establish the age of the formation^{1–4}; these are the BKT-2 tuff and the Kadada Moumou basalt. Other volcanic horizons have large detrital components or excess argon is suspected rendering them unsuitable for age determinations.

The age of the BKT-2 is interpreted as 2.88 ± 0.08 Myr, based on several K/Ar age determinations and fission track data from zircons^{1–4}. However, the age of a Kadada Moumou basalt is not clear. Repeated K/Ar analyses do not converge on a single age and age estimates show a very strong correlation with petrology. Walter³ identified three varieties of the basalt identified as A-1, A-2 and B type basalts yielding K/Ar ages of 2.7–3, 3.2–3.4, and 3.6 Myr respectively.

The type B basalt is presently represented by only one hand sample and although the age is probably quite reliable, because it is represented by only a single sample and because its chemistry is different from the A types, it is necessary to consider a possible interpretation that it is a xenolith from an older flow, or that it contains excess argon. There are problems with the type A basalts also, for they show a correlation between observed alteration and age. As might be expected, the A-1 type basalts which are the most altered give the youngest ages. However, Walter *et al.*⁴ prefer the 3.6-Myr age for the basalt. Figure 3 shows two possible interpretations of the polarity data as indicated by the various ages which may be attributed to the Kadada Moumou basalt.

The original interpretation^{1,2,7} assumes the A-1 age, 2.7–3.0 Myr, for the basalt. The two reversed zones are then correlated with the Kaena and Mammoth events within the Gauss normal epoch. This interpretation is consistent with the reversed magnetization of the Kada Moumou basalt.

A second interpretation (not shown) assumes the A-2 age, 3.2–3.4 Myr, for the basalt. This requires the upper reversed zone to represent the Kaena and Mammoth events. This is inconsistent with the lower reversal, in that the lower reversal which includes the reversed basalt would correlate with the base of the Gauss normal epoch. For this correlation to be true, the Kadada Moumou basalt and lower reversal would have to be the result of remagnetization. There is no evidence of remagnetization of the basalt and consequently this possibility is rejected.

The third interpretation which assumes a type B age for the basalt, 3.60 ± 0.15 Myr, requires the upper reversed zone to contain both the Kaena and Mammoth events. The lower reversal would correlate with the top of the Gilbert reversed polarity epoch. While satisfying most of the polarity data, the extent of the normal section below the reversed horizon poses a problem.

Table 1 Sedimentation rates implied by the different interpretations of the Hadar Formation

Stratigraphical interval	Magnetic interval	Stratigraphical thickness (m)	Interpretation 1		Interpretation 3a		Interpretation 3b		Depositional environment
			Age range (Myr)	Sedimentation rate (cm kyr ⁻¹)	Age range (Myr)	Sedimentation rate (cm kyr ⁻¹)	Age range (Myr)	Sedimentation rate (cm kyr ⁻¹)	
Middle KH	Upper reversal	35	2.92–3.01	39	2.92–3.15	15	2.92–3.15	15	Deltaic
Lower KH	Middle normal	40	3.01–3.05	100	3.15–3.40	16	3.15–3.40	16	fluvial
Middle DD	Lower reversal	25	3.05–3.15	25	3.40–3.80	6	No data	No data	Deltaic fluvial
Upper SH	Lower normal	70	3.15–3.40	28	3.80–3.90	70	No data	No data	Lacustrine
Base									Deltaic fluvial lagoonal base

To account for the absence of a reversed interval between the Cochiti and Nunivak events either the normal magnetization at the base of the section consists of the Cochiti event (which would require a very high sedimentation rate), interpretation 3a, or the base of the section has been remagnetized, interpretation 3b, which is shown.

The net sedimentation rates through the section can be used to examine the internal consistency of the various age interpretation for the basalt. Table 1 shows the net sedimentation rates for the stratigraphical section implied by the interpretations 1, 3a, and 3b. The sedimentation rates were calculated by dividing the thickness of the magnetozones by the duration of appropriate zone as given by Mankinen and Dalrymple⁸. The thicknesses are approximate in that the thickness of the Hadar Formation is variable in the study area. Most of the variation, however, is due to variation in thickness of the SH unit. The depositional environment of the Hadar Formation is given in the right column of Table 1; details of the development of the sedimentary model are given elsewhere^{9,10}. The sedimentation rates in a fluvio-lacustrine environment such as the Hadar Formation may be expected to be variable. However, the broad trends in sedimentation should vary with the sedimentary environment. During periods of fluvio-deltaic activity when large amounts of sediment are being deposited in the nearshore regions and deltaic facies are dominant, then relatively high sedimentation rates are possible (though unconformities could also be present). When there was a major transgression of the lake and lacustrine environments dominated, the sedimentation rate could be expected to be lower.

Interpretation 3a implies a sedimentation rate of only 6 cm kyr⁻¹ for the Upper SH to Middle Denon Dora but a minimum sedimentation rate of 70 cm kyr⁻¹ for the lower part of the section. While an unconformity could be present which could lower the apparent sedimentation rate, there is no evidence of

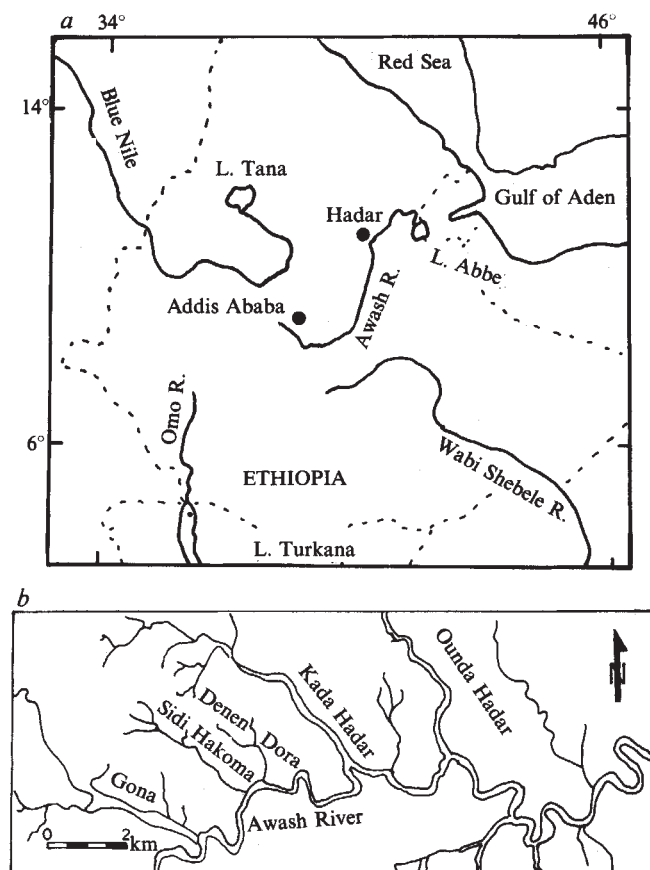
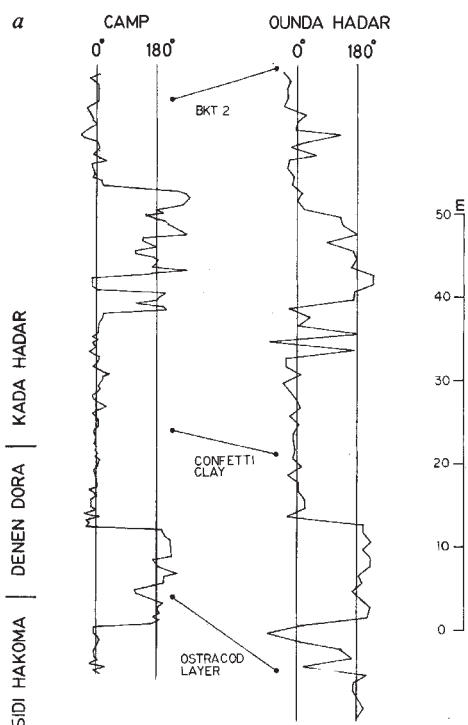


Fig. 1 Setting of the Hadar hominid site. *a*, Location of Hadar with respect to major drainage. *b*, Location of stratigraphical sections with respect to local drainage of the Hadar region.

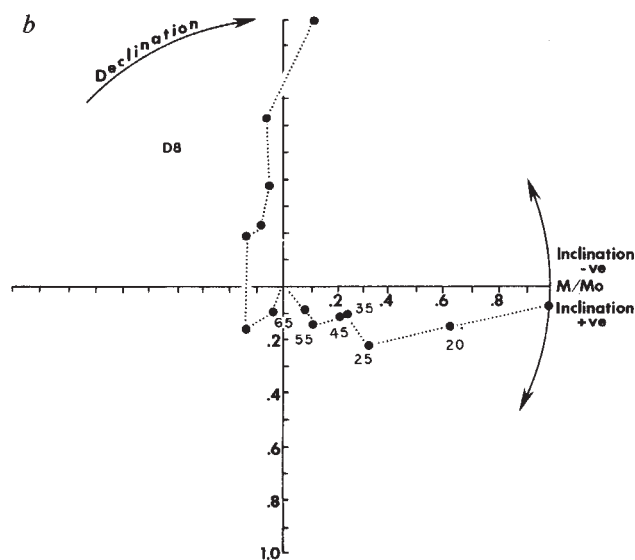
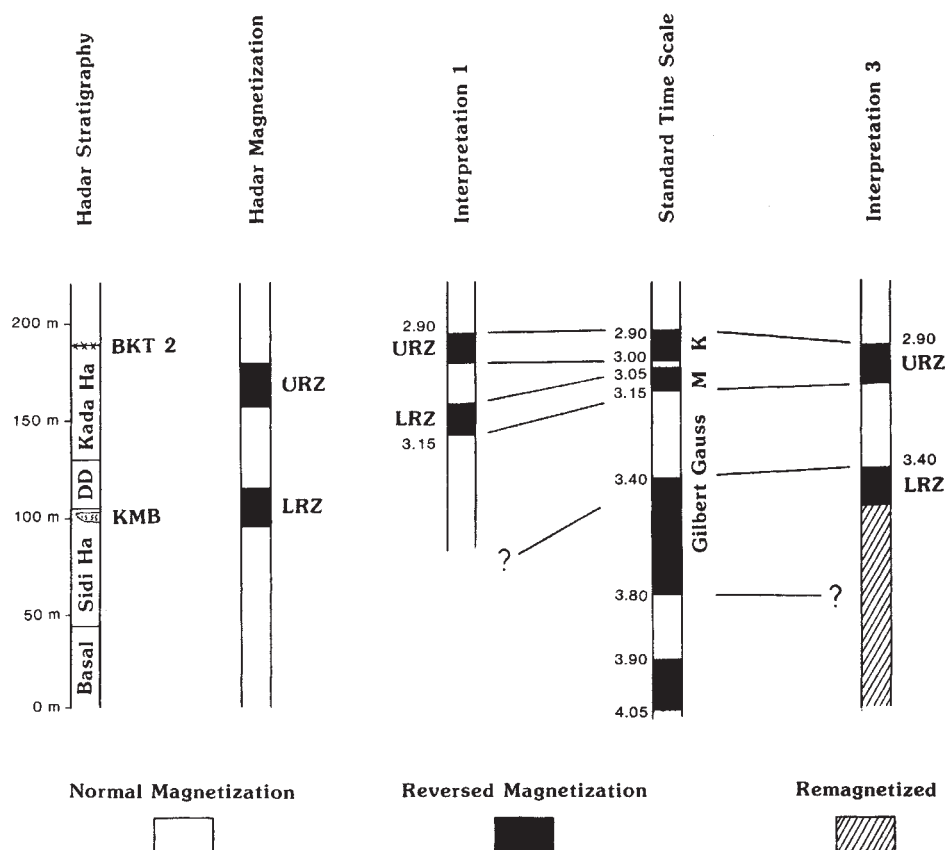


Fig. 2 *a*, Magnetic declinations plotted against stratigraphical position of the upper half of the Hadar Formation. Declinations are plotted from the upper half of the camp and Ounda Hadar sections. The lower half of both sections are normally magnetized. The magnetic directions are cleaned to 500 or 750 Oe. If the samples were still changing direction between 350 and 500 Oe then they were demagnetized to 750 Oe. The previous presentation of the camp section^{2,7} was at lower demagnetization stages. *b*, Stable end point diagram of a representative sample of the Hadar Formation. The declination and inclination of magnetization of the sample are plotted against normalized intensity of magnetization at each demagnetization stage. The demagnetization stages are listed in the diagram in mT. The first stage is NRM. A significant change in direction of the sample (a reversal) with high demagnetization fields is shown.

Fig. 3 The various interpretations of the Hadar magnetostratigraphy are shown with the 'standard' magnetostratigraphy and stratigraphy of the Hadar Formation. The standard ages for the Gauss and Gilbert are from ref. 8. K, Kaena; M, Mammoth events. The Hadar magnetization is from this and previous papers. The interpretations are as in the text. The schematic of the Hadar Formation is modified from Walter and Aronson⁴; the thicknesses of the Hadar Formation are variable, the thicknesses noted here are approximately the thicknesses of the members of the 1975 camp section.



a time gap large enough to reduce the rate to that of 6 cm kyr^{-1} . Also, the implied minimum sedimentation rate of 70 cm kyr^{-1} is considerably higher than the sedimentation rate implied for similar facies elsewhere in the section.

Interpretation 1 implies a relatively high sedimentation rate ($40\text{--}100 \text{ cm kyr}^{-1}$ for the fluvio-deltaic periods and low rate (2.5 cm kyr^{-1}) during the lacustrine period. The 28 cm kyr^{-1} rate for the base and SH is a minimum rate and thus not inconsistent with the higher rates for the similar facies higher in the section.

Interpretation 3b implies a sedimentation rate of $15\text{--}16 \text{ cm kyr}^{-1}$ for the upper part of the section. The sedimentation rate for the lower part is indeterminate due to interpreted remagnetization.

In the Omo Valley of southern Ethiopia and northern Kenya and the basins along Lake Turkana in Kenya there are hominid-bearing fossiliferous strata temporally equivalent to the Hadar Formation. Further constraints on the age of the Hadar Formation can be made by comparison of the Hadar Formation with those formations. Brown⁵ and Boaz *et al.*⁶ compared the Hadar Formation with the Omo and Turkana facies by geochemical and palaeontological means, respectively.

Brown compared the minor and trace elements chemistry of the Sidi Hakoma Tuff of the Hadar Formation with the Tula Bor Tuff of the Koobi Fora Formation, and its equivalents, Tuff B of the Shungura Formation, and Tuff U-10 of the Usno Formation. They are strikingly similar. He took this to indicate that they were the result of the same volcanic eruption. The correlation is consistent with the magnetostratigraphy. These tuffs are assigned to the lowest normal of the Gauss Normal epoch; thus the stratigraphical correlation implies that the lower reversed zone of the Hadar Formation is the Mammoth event, between 3.05 and 3.15 Myr.

Boaz *et al.*⁶ attempted a biostratigraphical correlation of the Hadar Formation with the Shungura and Usno formations. They compared the evolutionary stage of individual genera and species and made a statistical correlation of the Hadar fauna with a set of a faunal assemblages from the age range 2.9–

3.15 Myr. They found the Hadar Formation most similar to the Usno Formation, which has the older of the two faunas. They concluded that the biostratigraphy was consistent with the geochemical correlation made by Brown⁸. There are, however, some constraints on biostratigraphy, for the age of the Hadar Formation is from 2.9 to at least 3.15 Myr and perhaps 3.6 Myr. That is, the Hadar age range covers both of the standards compared and although most of the Hadar fauna are from the Denon Dora member, the correlation was based on the full faunal list.

The consistency of the polarity sequence of the two separate magnetostratigraphical sections after cleaning strongly suggests that the sequence is a true record of the magnetic field at the time of deposition of the strata and that there is minimal remagnetization. The age of the upper part of the section is reliably established as about 2.8 Myr. Using that as an anchor point the uppermost reversed zone is correlated with the Kaena event and the middle reversed zone is correlated with the Mammoth event. This is consistent with regional geochemical⁵ and biostratigraphical⁶ correlations. The net sedimentation rates implied by the correlation are consistent with the A type basalt ages and supports the original^{1,2,7} age estimates for the Hadar Formation and its important fossils.

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Adult-obtained pyrrolizidine alkaloids defend ithomiine butterflies against a spider predator

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Ithomiine butterflies, the original distasteful models in neotropical mimicry complexes, show warning coloration and are usually rejected by both vertebrate and invertebrate predators¹⁻⁴. Based on co-evolutionary theory^{1,5-7} and the *Danainae*-cardenolide example⁷, this protection has been ascribed to toxic compounds sequestered by larval Ithomiinae from their host-plants, usually poisonous Solanaceae⁸⁻¹⁰. However, although the giant tropical orb spider *Nephila clavipes* cuts out field-caught Ithomiinae from its web^{4,11} it readily eats freshly emerged adults. Solanaceae toxins are not found stored in adult Ithomiinae^{6,11} and when applied to the palatable¹ butterfly *Biblis hyperia* do not induce rejection behaviour in *Nephila* (Table 1). I show here that the protection of ithomiines against this abundant predator is due to dehydropyrrolizidine alkaloid monoesters and their *N*-oxides, absent from Solanaceae but sequestered by these butterflies as adults from flowers (mostly Eupatorieae) and decomposing foliage (mostly Boraginaceae)¹¹⁻¹³. These compounds are also important in the reproduction of the Ithomiinae^{11,14}.

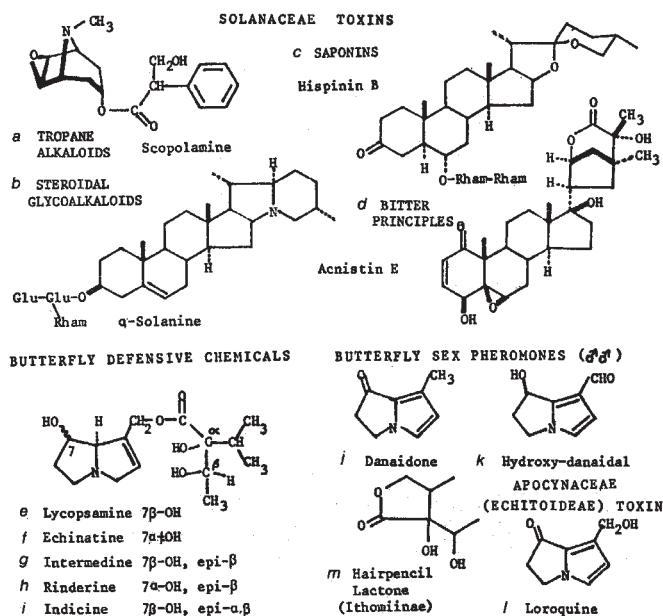


Fig. 1 Representative Solanaceae toxins (a-d)⁸⁻¹¹ and Danainae/Ithomiinae defensive and reproductive substances (e-m)^{11-16,21-24}.

Table 1 Chemical analysis and bioassay of Ithomiinae and their foodplants

Ithomiine tribes (b-l), representative genera (no of species analysed/total no. in genus) and PA content of individuals in typical populations (average % dry weight; male/female)	Fractions bioassay	Larval foodplants: family, tribe (I-XII or name), representative genera (no. spp. analysed)	Chemical compounds	Fractions, bioassay
(b) <i>Tithorea</i> *† (1/2)0.30/0.30; (c) <i>Aeria</i> † (3/3)2.3/1.6	Ex (+)	Apocynaceae: Parsonsieae (fresh leaves, roots, stems)		
(d) <i>Methona</i> *†‡ (4/7)0.031/0.026	Ex (+)	<i>Prestonia</i> (2), <i>Peltastes</i> (1), <i>Temnadenia</i> (1)	<i>eloqr</i>	Ex, 1, 2 (-)
(e) <i>Placidula</i> † (1/1)0.11/0.037	Ex (+)	Solanaceae (fresh leaves)		
(f) <i>Melinaea</i> * (5/10)2.8/1.1	Ex (+)	XII <i>Brunfelsia</i> (2)	<i>a?no</i>	Ex (-)
(g) <i>Thyridia</i> * (1/1)2.7/2.2, <i>Sais</i> * (1/2)3.6/1.7	Ex (+)	II <i>Datura</i> (1), <i>Brugmansia</i> (1)	<i>adnop</i>	Ex, 2 (-)
V <i>Mechanitis</i> *† (5/5)1.8/2.6 or 2.8/1.8, <i>Scada</i> (12/6)13.4/3.6;	Ex, 2, 3 (+)	VI <i>Solandra</i> (1); VII <i>Markea</i> (<i>Dyssochroa</i>) (1)	<i>as</i>	Ex, 1 (-)
(h) <i>Hypothyris</i> *† (6/16)2.2/1.5;	Ex (+)	Ia <i>Cyphomandra</i> (2), <i>Lycianthes</i> (2)	<i>bcnops</i>	Ex, 1 (-)
(j) <i>Dircenna</i> *† (3/7)1.7/0.88	Ex (+)	<i>Solanum</i> , subgenera <i>Brevantherum</i> , <i>Leptostemonum</i> , <i>Bassovia</i> and <i>Potatoe</i> (14)	<i>bcnp</i>	Ex, 1-4 (-)
(h) <i>Epityches</i> † (1/1)6.7/3.4;	Ex (+)	Ib <i>Physalis</i> (1), <i>Acnistus</i> (1), <i>Capsicum</i> (3)		
(i) <i>Ithomia</i> *† (4/21)5.4/2.0	Ex (+)		<i>acd</i>	Ex, 1-3 (-)
(k) <i>Prittwitzia</i> † (1/1)2.8/1.7, <i>Pteronymia</i> *† (5/41)9.0/3.3;	Ex, 2, 3 (+)	Ia <i>Solanum</i> , section <i>Geminata</i> (3)	<i>bcp</i>	Ex, 1, 2 (-)
(l) <i>Mcclungia</i> † (1/1)1.7/1.1, <i>Pseudoscada</i> † (3/6)4.8/2.2	Ex (+)	VIII <i>Cestrum</i> (2)	<i>bcops</i>	Ex, 1, 2 (-)
Adult foodplants of Ithomiinae, Danainae and Arctiidae, and larval foodplants of various species in the latter and other moth families as well as some Diptera	Ex (+)	Boraginaceae: <i>Heliotropium</i> (3) (leaves)	<i>his</i>	Ex, 2 (+)
		Compositae: Eupatorieae (leaves)		
		<i>Adenostemma</i> (2), <i>Trichogonia</i> (1), <i>Eupatorium</i> (8)§	<i>e - i, ors</i>	Ex, 2, 3 (+)

Complete species lists and analytical results are given in ref. 11. Over 2,500 individual butterflies in Danainae (3 genera, all 6 South American species) and Ithomiinae (32 of 34 Brazilian or 51 neotropical genera, 82 species) were analysed and shown to store PAs. In most individuals, 50-65% of the PA was *N*-oxide and 2-5% dihydropyrrolizine (type *k*), with ketones (types *j* or *l*) also detected. Unspecified amounts of PAs (*e* + *g*), previously found in *Oleria* and *Hypomenitis*, were considered as precursors of pheromone *m*, isolated from male hairpencils of *Ithomia*, *Pteronymia*, *Godyris*, *Hypomenitis* and *Greta*¹⁴. A total of 42 species of larval foodplants (in 19 genera), extracted and fractionated in parallel with their herbivores, always gave negative assays with *Nephila* except with very heavy loads on the *Biblis* (>10 mg; became negative upon dilution). Over 400 blind bioassays were performed using over 100 *Nephila*, in triplicate (if +) or duplicate (if -), with code-numbered total extracts (Ex) or fractions (1-neutral; 2-alkaloidal; 3-polar; 4-apolar). Letters for chemical compounds refer to Fig. 1, plus *n*-other alkaloid classes, *o*-phenolic glycosides, *p*-pungent oils, *q*-cardenolides, *r*-lower terpenes and *s*-alkaloid *N*-oxides. Ithomiinae and Solanaceae tribe letters and numbers are in approximate evolutionary order^{8,10}; note the apparent lack of 'parallel diversification' type co-evolution between the genera of these herbivores and their host-plants^{5,10,11}.

* Eggs of these species showed from 0.1% (*Methona*) to 9% of dry weight as PAs.

† Mature larvae of these taxa are aposematic in colour-pattern.

‡ Recently emerged adults of 13 species in these 10 genera, of both sexes, reared in the laboratory from eggs or larvae and denied access to PAs, were consumed without hesitation by *Nephila* which in many cases had just cut out a field-captured adult of the same species and sex. Mattocks-Bingley assay^{25,26} showed no PAs in these and in many other recently emerged adults.

§ Flowerheads of several abundant Brazilian species of *Eupatorium* contain 1-4% of a single isomer of PA (*k-o*) and the corresponding *N*-oxide, and thus represent important new sources for these compounds, some of which are now in advanced clinical testing as antitumour agents.

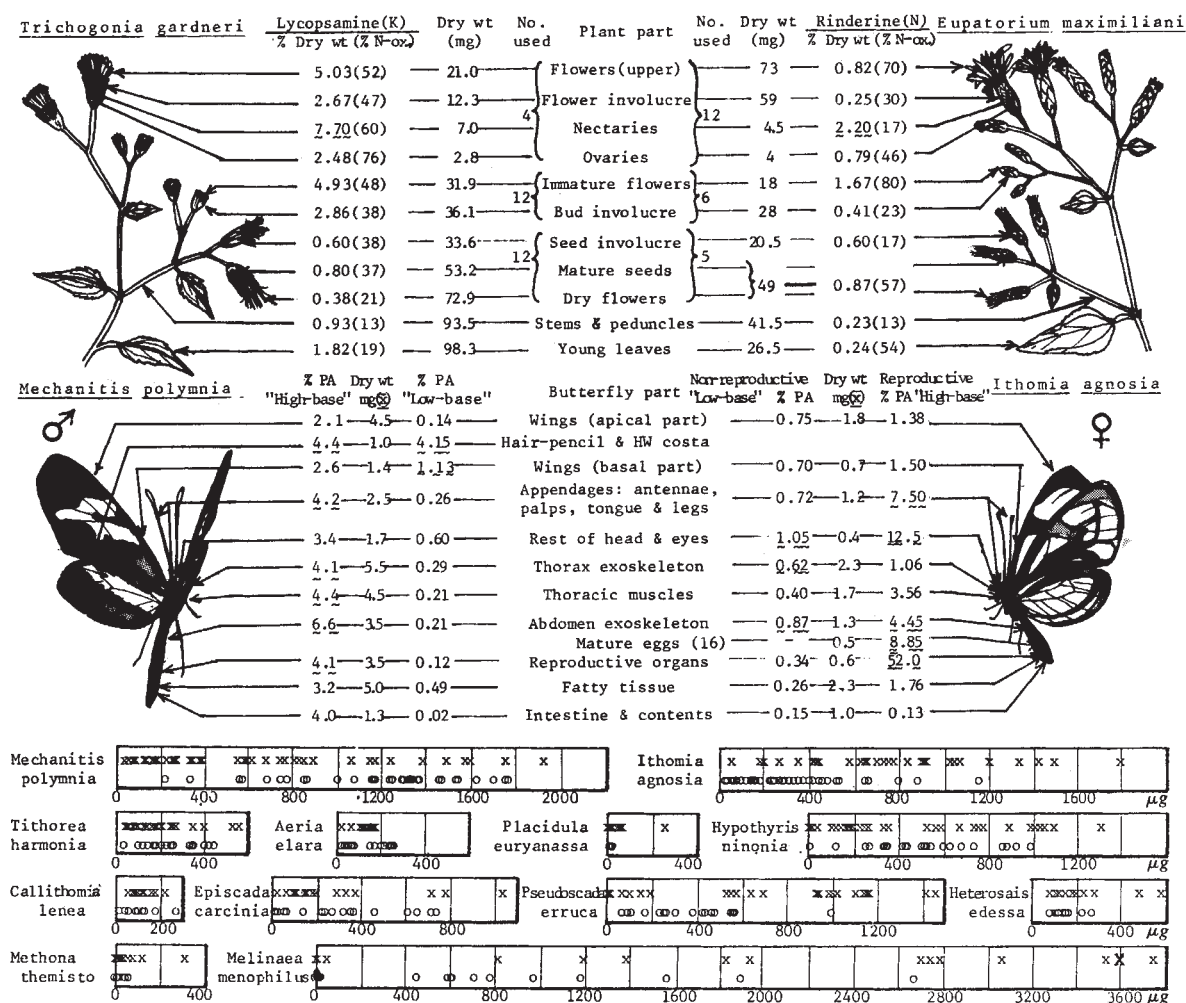


Fig. 2 Distribution of PAs in two species of Eupatorieae commonly visited by Ithomiinae (upper), and (lower) within and between individual adults of typical Ithomiinae (see Table 1 for further data and chemoassay details). High-base non-reproductive and low-base reproductive females of *Ithomia agnosia* were also encountered. The thorax tended to show low N-oxide content (<50% of PA) and detectable dihydropyrrolizines in both species dissected, while abdominal contents usually had low PA in non-reproductive (winter dry season) individuals. The variation in total PA between adults in a given population (males as crosses on upper line, females as circles on lower in each graph) was random or polymodal, indicating a wide 'palatability spectrum'. Individuals near the lower end, usually very young, would be true 'automimics' in the population and should be readily eaten by *Nephila* (Fig. 2). The patterns suggest opportunistic accumulation of alkaloid by each individual as various sources become available to the population, up to a maximum of over 13% of dry weight (at least in *Napeogenes*, *Ithomia* and *Pteronymia*; *Scada* males captured near *Eupatorium* flowers averaged over 13% and one showed over 20%), or 5.8 mg (in a male *Melinaea mneme*). Recently emerged adults (laboratory-reared or field-captured) showed no PA; a highly positive correlation was found in males, but not in females, between PA content and estimated age. Typical spreads, concentrations and male-female patterns in PA storage are shown by *Ithomia*. A few genera (*Tithorea*, *Placidula*, *Callithomia*, *Heterosais*, and especially *Methona*) show low accumulation in adults. *Tithorea*, *Aeria*, *Mechanitis*, *Rhodussa* and *Hypothyris* may show greater average storage of PAs in females than in males in some populations, but highest concentrations are always found in reproductively active males. Males attracted to baits (PA sources) and females attracted to displaying males showed low concentrations, suggesting an 'appetite effect'. In a notable case of a mating pair of *Melinaea menophilus*, the male was about to transfer 1.7 of his 3.6 mg of PA in a spermatophore (a small sac with less than 10% of his dry weight) to the virgin female who contained no PA. Other courting or mating males of seven genera analysed showed similar channelling of PA into spermatophores (up to 23% of dry weight or 50 times the concentration found in the rest of the organism), supporting a suggestion by L. Gilbert that females, rarely found on PA sources, get most of their alkaloid from males during mating. As they mate opportunistically and frequently, and place much of their PA on the eggs they lay, their content does not correspond so well with age.

Many pharmacological, physiological and ecological activities of dehydropyrrolizidine alkaloids (PAs) are known¹⁵. Danaine butterflies (sister-group to the Ithomiinae¹⁶) and arctiid moths sequester PAs from larval foodplants, or as adults obtain them from the same sources as do the Ithomiinae, using them as physiological regulators, pheromone precursors and defence substances^{12,13,17-20}. In Danainae that store cardenolides erratically from larval food^{21,22} PAs have often been suggested as alternate predator-defence compounds^{13,21-24}. In *Utetheisa* (Arctiinae), larval-sequestered PA diesters protect adults against *Nephila clavipes*¹⁸. Some male Ithomiinae were suggested to use adult-sequestered PAs as hairpencil pheromone (Fig. 1m) precursors¹²⁻¹⁴, but these butterflies have usually been assumed to

sequester Solanaceae toxins for defence¹.

Methanol-water extracts, prepared by rapid vigorous homogenization of fresh leaves or flowers of larval and adult foodplants and fresh butterflies, were separated into medium-polarity neutral, alkaloidal and highly polar fractions, containing different classes of secondary compounds (Fig. 1). Further extraction with ethyl acetate gave an apolar fraction. Extracts and fractions were characterized by chromatography and ¹H-NMR spectra, and bioassayed with large populations of *Nephila* in ithomiine 'pockets'^{2,3}. A small amount (corresponding to that extracted from 0.5-1.5 butterflies, 0.5 g fresh leaf of larval food-plant or 0.05 g fresh leaf or flowers of adult food), in aqueous solution or suspension, was applied with a soft brush onto the

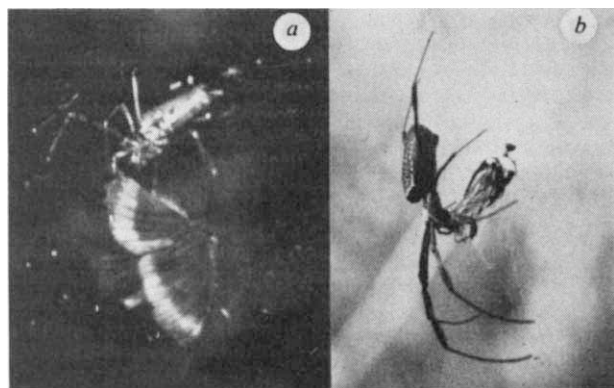


Fig. 3 *a*, *Nephila clavipes* cutting out a *Biblis hyperia* treated externally with the total MeOH-H₂O extract of one *Mechanitis lysimnia* adult. *b*, *Nephila* eating a freshly emerged *Pseudoscada erruca* adult, reared from the egg on *Cestrum laevigatum*.

body and wing bases of the common nymphaline *Biblis hyperia*. This was thrown into the web of a mature, non-satiated *Nephila*, which either readily ate the *Biblis* or cut it out (Fig. 3).

All *Biblis* treated with Solanaceae leaf extracts or fractions, or apolar or neutral fractions of Ithomiinae, like newly emerged adult Ithomiinae except *Aeria* (Apocynaceae-feeder), were accepted by *Nephila* (Table 1). In contrast, all total extracts and alkaloidal or polar fractions of field-captured Ithomiinae and their principal food sources caused *Nephila* to cut *Biblis* out just like intact wild Ithomiinae. All active extracts and fractions contained one or more PA monoesters (Fig. 1*e-i*), free or *N*-oxides. When freshly emerged adult Ithomiinae were fed pure PA *N*-oxide (0.3 mg) they became unpalatable to *Nephila* after about 1 h.

The distribution of PAs in Ithomiinae and food sources, measured by a modified Mattocks-Bingley assay^{25,26}, corresponds to their ecological and reproduction roles. The Eupatorieae, often pollinated by Ithomiinae¹², show highest concentrations in the flowerheads and especially nectaries, while the butterflies concentrate the alkaloids in the tegument (where the predators will immediately sense them), reproductive tissues and eggs (Fig. 2).

Most Ithomiinae found on PA sources are males^{11,12}. In chemoassay, males of most genera show a broader range of PA content and higher extreme accumulation than females (Table 1, Fig. 3). Reproductively active males channel up to half their total PA into spermatophores that are transferred to females, who accept multiple matings and may obtain much PA in this fashion (Fig. 2).

Thus freshly emerged adult Ithomiinae, which unlike many Danainae and Arctiidae bear no accumulated toxins from larval foodplants, must find in their environment the PAs used in defence and reproduction. This necessity determines many chemical, physiological, behavioural, and morphological traits of the adult insects and of the plants they pollinate. Selection probably favours the obtaining of such essential compounds from a source stabilized by mutualism, rather than from the solanaceous larval foodplants, whose diversified chemical leaf constituents are constantly changing in response to the antagonistic setting and do not offer protection against *Nephila*, a major potential predator of Ithomiinae.

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Fibre type composition of single motor units during synapse elimination in neonatal rat soleus muscle

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Skeletal motor neurones innervate the specialized 'types' of fibres comprising most mammalian muscles in a characteristic fashion: each motor neurone forms a 'motor unit' by innervating a set of fibres all of the same type¹⁻⁴. Because the type expression of adult muscle fibres is plastic and apparently controlled by their innervation, each motor neurone is thought to impose a common type differentiation on all the fibres in its motor unit⁴⁻⁶. However, the situation in developing muscles cannot be this simple. Muscle fibres in neonates receive synaptic input from several motor neurones and achieve the adult, single innervation only after a period of 'synapse elimination'^{7,8}. Despite this polyneuronal innervation, differentiated fibre types are present in neonatal muscles⁹⁻¹⁴. This means either that the motor neurones polyneuronally innervate fibres in a random fashion and type expression is not determined by innervation or that the polyneuronal innervation is ordered in such a way that each fibre could receive unambiguous instructions for type differentiation. We have investigated these possibilities here by determining the fibre type composition of motor units in neonatal rat soleus muscle. We find that even during the time of polyneuronal innervation each motor neurone confines its innervation to largely one of two fibre types present in the muscle. Therefore, some mechanism during early development segregates the synapses of two groups of soleus motor neurones onto two separate populations of soleus muscle fibres.

We examined the soleus of Wistar rats at two postnatal ages—at 8 days, when every fibre is innervated by at least two motor neurones, and at 16-17 days, by which time synapse elimination has resulted in virtually every fibre being singly innervated⁸. In agreement with previous work⁹⁻¹¹, we found two distinct fibre types in soleus cross-sections stained for myofibrillar ATPase activity (Fig. 1). At both ages muscles showed a mosaic of lightly and darkly stained fibres. Because these neonatal muscles contain a number of novel embryonic and neonatal myosin

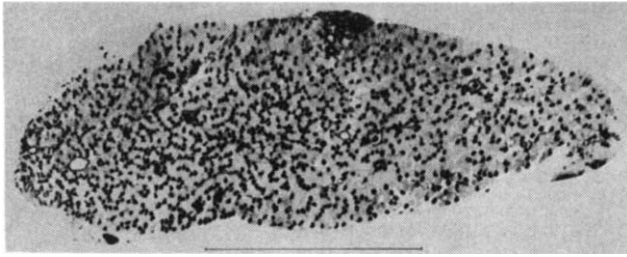


Fig. 1 Cross-section of a 16-day-old rat soleus muscle stained for myofibrillar ATPase activity after alkaline preincubation at pH 10.4 according to the method of Guth and Samaha³⁴. Two muscle fibre types are visible, termed light and dark according to staining intensity. Scale bar, 1 mm.

isozymes^{13,15-17}, there is some uncertainty as to the exact adult fibre types to which each staining pattern corresponds. Although the light fibres presumably become type I fibres and the dark fibres type IIA fibres^{9,10}, we refer to these fibre types as 'light' and 'dark' based on staining intensity. Similar light/dark differentiation was seen at both ages (Figs 2a, c, 3a). Moreover, the total number of fibres and the proportion of each type appeared unchanged, despite the loss of over 50% of the synaptic connections in the muscle between 8 and 16-17 days.

To determine how single soleus motor neurones innervate these two fibre types, we used the glycogen depletion technique¹⁸⁻²⁰ to mark the fibres in a motor unit. The basis of this method is that stimulation of a single motor neurone activates the fibres comprising its motor unit and, in conditions of muscle anoxia, forces these fibres to utilize their glycogen stores. By staining muscle sections for glycogen, these fibres can be identified by their absent or weak coloration.

The procedures outlined by Kugelberg¹⁹ were used for isolating and stimulating a single soleus motor axon in anaesthetized rats. Fibres in the motor units of stimulated axons were easily recognized in sections stained with the periodic acid-Schiff method²¹ (Fig. 2b, d). Counts of depleted fibres showed that motor units at 8 days contained two to three times more fibres than those at 16-17 days (Fig. 3b), implying that the average muscle fibre at 8 days receives suprathreshold innervation from two or three motor neurones. These counts also offer some assurance that the depletion method is marking a large fraction of the fibres in units, as the present measurements are in reasonable agreement with estimates of motor unit size based on tension measurements (140 fibres at 16-17 days²² and 340 fibres at 8 days⁸). Thus, glycogen depletion reaffirms the polyneuronal innervation of neonatal muscles and the decrease in motor unit size accompanying synapse elimination^{8,23,24}. This decrease in unit size occurs as each motor neurone reduces the number of fibres it innervates, presumably as a result of competition among the terminals at each end plate⁸.

The fibre type of each depleted fibre was determined by identifying the same fibres in two adjacent muscle sections, one stained for glycogen and the other for ATPase activity (Fig. 2). By examining complete cross-sections in this fashion, the fibre type composition of motor units was determined (Fig. 4). The striking finding is that motor units at both ages were largely homogeneous. Given the proportions of light and dark fibres present in soleus, a random innervation would be expected to yield units containing a mixture of about 55% light and 45% dark fibres. However, even at 8 days units consisted of at least 72% of one fibre type, and three of the five units examined were >90% homogeneous (Fig. 4). As motor neurones primarily innervate either only dark or only light fibres at day 8, most of the polyneuronal innervation of dark (or light) fibres must come from neurones which form motor units of the dark (or light) type. Furthermore, because of this predominance of synapses

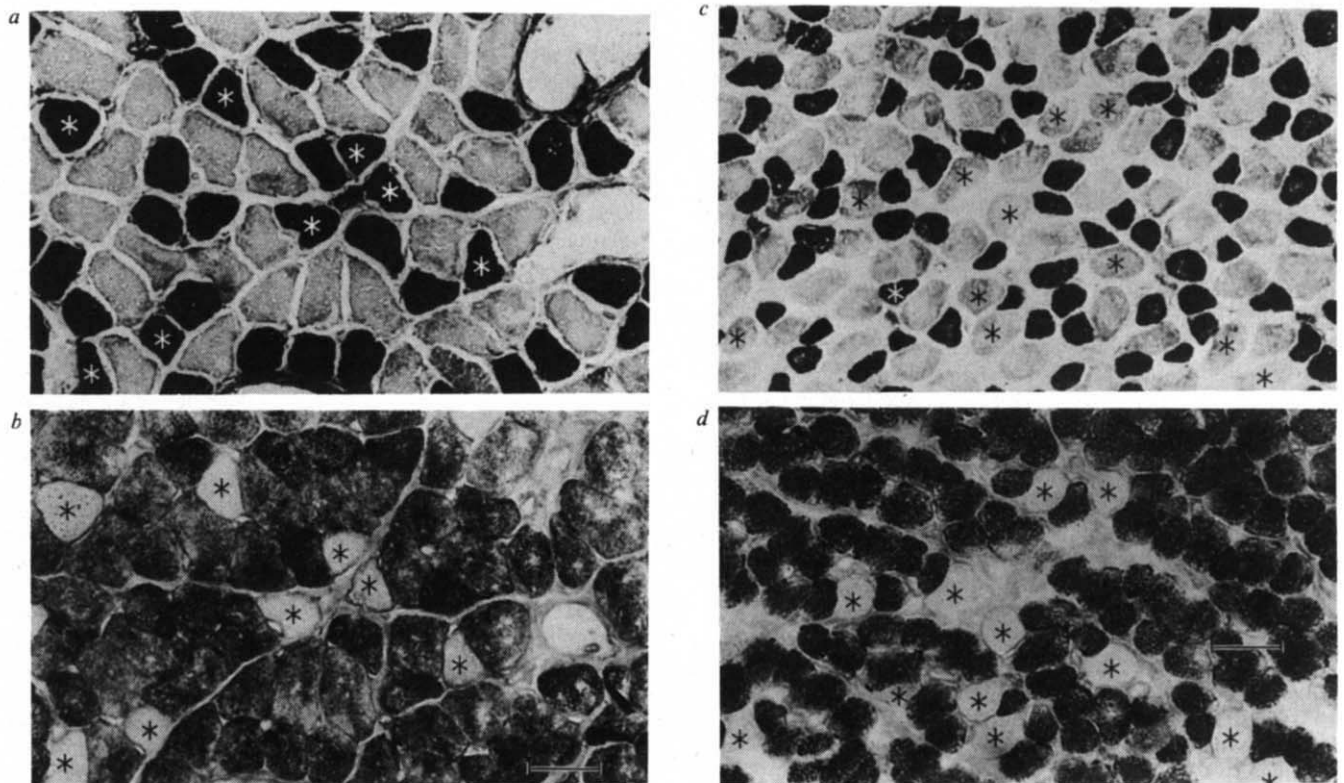


Fig. 2 Muscle cross-sections stained for myofibrillar ATPase activity after alkaline preincubation (a, c) and for glycogen (b, d). Adjacent sections stained by each method are shown for two muscles following stimulation of a single soleus motor axon. a, b, Portion of a motor unit from a 16-day-old rat. c, d, Portion of a motor unit from an 8-day-old rat. Asterisks mark glycogen-depleted fibres and the corresponding fibres stained for ATPase. Scale bars, 25 μ m.

placed on 'appropriate' types of fibres, most of the synapse loss between 8 and 16–17 days occurs from appropriate as opposed to inappropriate fibre types.

The most intriguing finding is the segregation of innervation by two groups of soleus motor neurones. One obvious explanation of this segregation is a selective synapse formation between predetermined types of neurones and muscle fibres. Indeed, this type of selectivity has been previously proposed for other neuromuscular systems^{25,26}, and certain recent findings support the existence of some type of predetermination of fibre type^{12,27–29}. Other observations, however, militate against such selectivity. First, reinnervation of adult fibre types is nonspecific and fibres mismatched with their innervation are gradually transformed in fibre type³⁰. Second, the differentiation of one type of fibre (slow, type I) seems to be neurally induced, as this type is absent in muscles denervated at birth^{11,27,31}. Moreover, there are certainly alternative explanations of the segregation which do not require a predetermination of muscle fibre types. In fact, the observed segregation of innervation is the result required if fibre types were to be dictated by instructions from innervating axons during polyneuronal innervation.

How might the segregation occur in the absence of predetermined fibre types? Two preexisting types of motor neurones might segregate their synapses onto separate naive muscle fibres as a result of an interaction among nerve terminals either during synaptogenesis or during a subsequent period of synapse elimination preceding 8 days. An alternative suggestion made by Rubinstein and Kelly¹² involves the timing of synaptogenesis

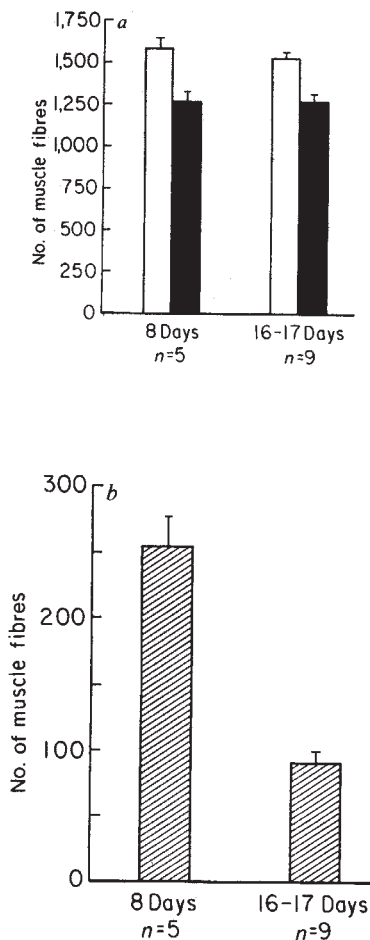


Fig. 3 Changes in muscle fibre type composition and motor unit size between 8 and 16–17 days. *a*, Number of dark (filled bars) and light (open bars) fibres found in muscles of each age. *b*, Numbers of muscle fibres comprising single motor units as determined by glycogen depletion. Vertical lines represent s.e.m.

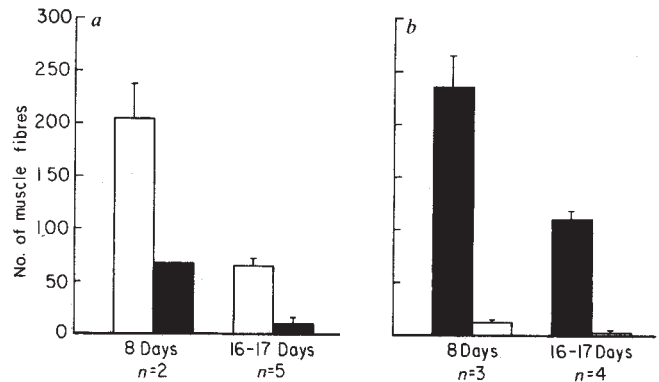


Fig. 4 Fibre type composition of motor units in soleus muscles at 8 and 16–17 days. Numbers of fibres of the dark (filled bars) and light (open bars) types are shown for motor units at each age. *a*, Units composed predominantly of light fibres. *b*, Units composed predominantly of dark fibres. Vertical lines represent s.e.m. (No vertical line appears for two units in the 8-day muscles in *a* which each contained 66 dark fibres.)

and myogenesis: if two groups of motor neurones arrive in the muscle at different times, coinciding with separate waves of myogenesis, these neurones could be constrained to innervate different sets of muscle fibres polyneuronal. Exactly how muscle innervation becomes segregated is unknown, but it is now evident that the generation of homogeneous motor units does not await the single innervation of muscle fibres.

We acknowledge that H. Gordon and D. Van Essen have reached similar conclusions to those reported here by their study of the contractile properties of motor units in neonatal rabbit soleus muscle (ref. 32 and work in preparation).

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Is stepwise sarcomere shortening an artefact?—a response

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Like any other signals, optical signals from muscle fibres inevitably contain noise. The noise here is of predictable character. Because the structure of muscle is periodic, or almost so, translational movement of an illuminated fibre will inevitably give rise to a periodic noise fluctuation as striations pass across the optical field. The frequency of this fluctuation should be linearly related to the speed at which the fibre translates. By mistaking this noise component for the signal, Altringham and colleagues¹ have amply succeeded in confirming this expected relationship, but they have left unanswered the question of the source and nature of stepwise shortening. I show here that: (1) by having obtained records in conditions in which the noise dominated, Altringham *et al.* have inadvertently confused the steps with the translation-induced fluctuation; and (2) when records are obtained using a method designed specifically to circumvent the effects of translation, the steps still remain in evidence.

Consider first the authors' Fig. 1 (ref. 1). To evaluate their method of analysis, consider, for example, the record labelled '2.72 μm ' in Fig. 1b. This record is not unlike many we have obtained and published^{2,3}. How many steps are there? I identify one pause (of moderate 'quality'), and hence two steps. The authors, however, have mistakenly confused the high-frequency fluctuations superimposed on the trace for the steps. Consequently, they counted 47 instead of 2. Records such as these gave the mid- to high-frequency points on their Fig. 1a, drawing the authors towards the inappropriate conclusion that the step frequency was linearly related to translation velocity. Instead, what they have shown is that it is the fluctuation that is linearly related to translation velocity. Had the authors plotted step frequency instead of fluctuation frequency, the resulting scatter diagram would have led them to an opposite conclusion to the one they drew.

The authors' Fig. 2 (ref. 1) illustrates the results obtained with the phase-locked loop (PLL, striation imaging) method. Consider the first (18-4-83) of the two experiments. As waveforms for this day's experiment were not shown by the authors, I have reproduced representative samples here in Fig. 1a, and show comparable records taken from our recent experiments in Fig. 1b. Note that the noise levels are 20–50 nm and 2–3 nm respectively. A step size of 10–20 nm, characteristic of our records, would therefore be buried in their noise. The authors, once again, appear to have studied a fluctuation.

Such fluctuations have, of course, been of concern to us. The PLL method inherently generates translation-induced fluctuations⁵, but we have demonstrated that with signals of reasonable quality, their amplitude remains 2 nm or less⁴. However, we have also obtained our share of records as noisy as those in Fig. 1a when one of a number of conditions had not been carefully controlled. Inevitably, the translation-induced fluctuation becomes the dominant feature of such records and masks the actual sarcomere shortening signal. While we ordinarily discard such records, Altringham *et al.*¹ have capitalized on them and have elegantly confirmed that the translation-induced oscillations correlate well with translation ($r = 0.999$).

In the second of the two PLL experiments, illustrated in the authors' Fig. 2b, Altringham *et al.* appear to be dealing with genuine sarcomere-length signals. However, their protocol ensures that the linear relationship will be found. In this second experiment, observation was restricted to a single region, while the moving end of the fibre was released at each of various speeds. Increasing the speed of release brings with it two (correlated) effects: it increases the local sarcomere shortening veloc-

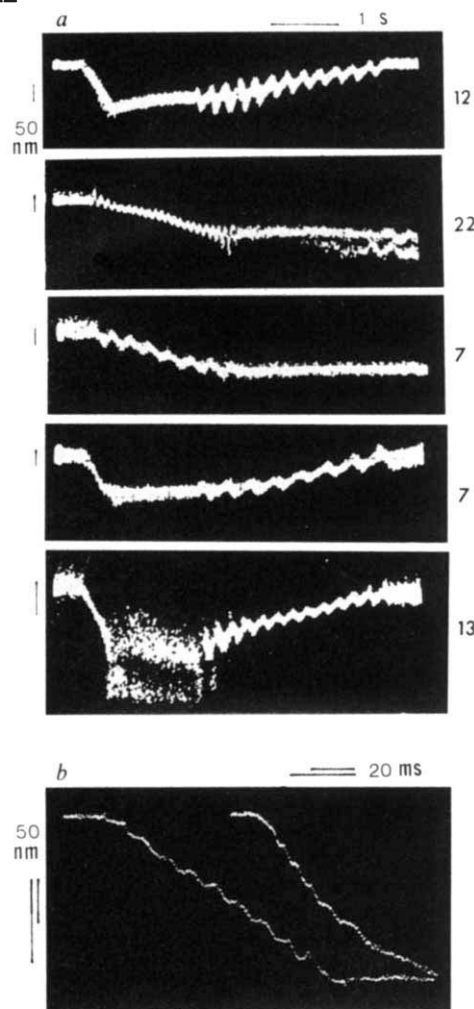


Fig. 1 Time course of sarcomere shortening measured with the phase-locked loop (PLL) imaging method. The five traces in *a* were obtained in the experiment of 18-4-83 and were kindly provided by Altringham *et al.*¹. (These are not worst-case records; peak-to-peak noise averaged among all 20 records was 32.5 nm, in the range of the five shown here.) Time bar calibration refers to all but the third panel, which is 200 ms. The number of oscillations counted by Altringham *et al.* is given at the right of each panel. The traces in *b* are representative of those obtained in experiments on unstimulated (left) and stimulated (right) frog toe fibres in this laboratory. They illustrate the character and size of the steps that we have measured^{4,15}. Note that steps of this size would probably be submerged in the noise of the upper set of records. Also note the vast differences of time scale between *a* and *b*.

ity, causing the steps to occur more frequently, and it also increases the local translation velocity. Thus, a linear relationship between step frequency and translation velocity is an inevitable consequence of their protocol. Further, since the translation velocity will increase from the fixed to the moving end, the slope of this linear relationship is not unique; it will depend on the choice of region to be studied. The particular value of slope found in this experiment is therefore without significance.

Finally, with regard to the authors' Fig. 3, we previously published essentially identical experiments except with 1,000 times higher time resolution. We showed that in regions which did not translate excessively, the first-order profile remained steady during the pause³. This convinced us that the pause represented a truly stationary period. Our conclusion did not imply that changes in first-order profile could not accompany the pause. Clearly, in the experiments of Altringham *et al.*, where substantial translation was an essential element in the protocol, and where only those diffraction patterns with 'less than

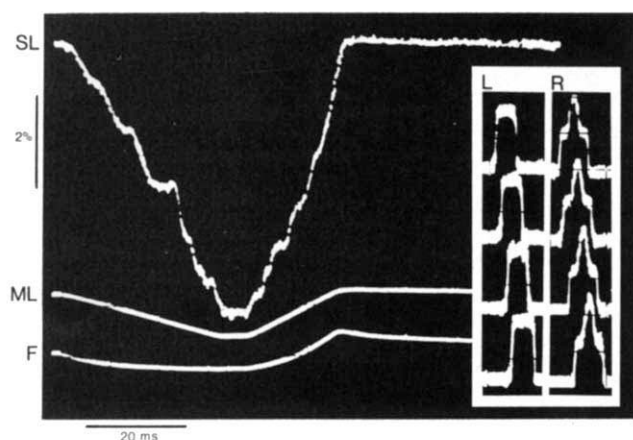


Fig. 2 Time course of segment shortening in an unstimulated single fibre of frog tibialis anterior muscle⁶. SL, segment length; ML, muscle length; F, force. Initial segment length was 515 μm . The segment is defined by the spacing between two hairs, nominally 10 μm in diameter and 80 μm in length, mounted on the upper surface of the fibre, perpendicular to its long axis. The image of the section of fibre containing the two hairs was split such that one hair was projected onto one photodiode array, while the other was projected onto a second array. A processing circuit computed the area under the image of each marker, from which the respective median locations were computed. The difference between median locations gave the segment length. Thus, the method is insensitive to translation. Of 17 fibres that gave usable segment length signals, 11 showed distinct, repeatable steps. The two inset panels show the intensity profiles of the left (L) and right (R) markers at four instants (top to bottom) spanning the longest shortening pause. They demonstrate that spurious fluctuations of intensity profile of either marker were not present, and therefore could not have given rise to an artefactual pause. Edman *et al.*⁸, using a similar method, also observed pauses in some records of segment length change (see their Fig. 8).

optimum quality' could be used, the detection of such changes is not at all surprising.

Thus, by using (1) noisy PLL signals, (2) weak diffraction patterns and (3) protocols in which translation was emphasized, Altringham and colleagues have set their attention on signals dominated by noise. In so doing, they have obtained clear proof that the noise arises out of fibre translation. It is unfortunate that the superficial resemblance between the translation-induced artefact and the stepwise shortening signal has given rise to this confusion. In fact, signal and noise are quite distinct. In the optimized conditions that we have been trying to realize since our early experiments (the reverse of (1)–(3) above), patterns such as those of Fig. 1b have begun to emerge consistently and serve to define the phenomenon. In such low-noise records, the shortening waveform approaches a true staircase, with clear pauses and regular cascades of steps of predictably distinct size^{4,15}. By contrast, the fluctuations shown in the authors' waveforms are of broadly variable size and character. The resemblance is thus only superficial.

Nevertheless, in light of the potential for confusion, we have designed a simple experiment to determine whether steps could still be observed in conditions in which the effects of translation were circumvented⁶. Using a segment-length detection method similar in principle to that developed by Gordon, Huxley and Julian⁷, we have observed stepwise shortening regularly in both stimulated and unstimulated fibres (Fig. 2). Similar waveforms have been found with other segment-length methods in three laboratories (refs 8, 9 and T. Tameyasu and H. Sugi, in preparation). We conclude, therefore, that steps arise independently of translation.

The phenomenon of stepwise shortening is not easily reconciled with commonly accepted views of contraction, as the authors state¹. This is perhaps why the challenge by Altringham

et al. is not unique; it represents the fourth in a series, each laying claim to identification of the long-sought artefact^{1,10–12}. Yet the phenomenon remains robust. It has now been observed in a variety of laboratories using four classes of methodology: laser diffraction^{2,3,10,13}, on line imaging of the striations^{4,15}, high-speed cinemicrography (ref. 14 and H. Sugi and M. Toride, in preparation), and various segment-length methods (refs 6, 8, 9 and T. Tameyasu, T. Yoyoki and H. Sugi, in preparation). Although some unsuspected artefact may yet remain, I know of no other phenomenon that has been confirmed by as many distinct methods as this.

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POLLACK says that the fluctuations illustrated in Fig. 2b of the article by Altringham *et al.* 'appear to be genuine sarcomere-length signals'. However, the fact pointed out by Altringham *et al.* that 'one oscillatory cycle corresponds to the translation of one sarcomere past a given point in the optical field' makes it practically certain that in this case the fluctuation is an instrumental artefact. The existence of 'noise' fluctuations of this kind is admitted by Pollack. Its origin in experiments using the PLL device is clearly seen in Fig. 1 of Pollack's ref. 4: the crude frequency signal fluctuates at one cycle per sarcomere in the image, and this fluctuation will be eliminated only if the number of sarcomeres m over which the signal is averaged is an integer (n). m depends on sarcomere length, and when $m = n + 1/2$, the peak-to-peak fluctuation in the average as the image moves across the field is $A/\pi m$, where A is the peak-to-peak amplitude fluctuation in the signal being averaged. With the values given in Pollack's ref. 4, this comes to 3.6 nm, which is close to the amplitude of the deviations from a smooth curve in Pollack's Fig. 1b and in Fig. 2b of Altringham *et al.*, both of which are taken by Pollack to represent genuine changes of sarcomere length.

These reasons for believing those fluctuations to be artefactual are not upset by Pollack's claim that 'a linear relationship between step frequency and translation velocity is an inevitable consequence of their [Altringham *et al.*]'s protocol': true, but the protocol does not ensure one cycle per sarcomere, which would be an unlikely coincidence unless the fluctuation is an instrumental artefact. Pollack's suggestion that the slope of the relationship 'will depend on the choice of region to be studied' is contradicted by the observation of Altringham *et al.* that 'the frequency of the oscillations appears to be determined only by the translation velocity of the fibre at the point observed, and not by the rate of change of sarcomere length'.

I conclude that Altringham *et al.* are right in claiming that the rather regular fluctuations often seen with the PLL method

are an artefact. The 'one pause' claimed as real by Pollack lasts for about 10 sarcomeres of fibre displacement, and may therefore be simply the consequence of a difference of sarcomere length between two regions in the fibre.

Details of the 'simple method' referred to by Pollack, with which his Fig. 2 was obtained, have not yet been published (his ref. 6 is 'in the press'), so there is no way of telling to what errors it is subject. There are many possible sources of error; for example, sideways movement of the fibre if the markers are not perpendicular to the fibre axis, or vertical movement of the fibre if the light beams used for following the two markers are not parallel to one another.

Transposition bursts in genetically unstable *Drosophila melanogaster*

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A genetically unstable strain of *Drosophila melanogaster*, ct^{MR2} , which appeared in the course of hybrid dysgenesis¹, was found to contain an unstable mutation in the *cut* locus, resulting from an insertion of the mobile element *mdg4* (ref. 2). Multiple genetic events, probably resulting from transposition, were found in this strain and its derivatives³. We have analysed the localization of various mobile elements within the X-chromosome of the ct^{MR2} strain and its derivatives. We report here a dramatic change in the localization of mobile dispersed genetic elements *mdg1*, *mdg2*, *mdg3*, and *copia*, FB- and P-elements within the X chromosome. Up to 20 independent transposition events were detected. As the localization of mobile elements within ct^{MR2} and within each substrain is invariant, we conclude that all transpositions occurred simultaneously in one and the same germ cell. Such 'transposition bursts' may have an important role in evolution.

The genetically unstable strain ct^{MR2} gives rise to several stable and unstable ct^+ reversions and novel *ct* mutations^{1,3}. These are frequently accompanied by other unstable mutations. All *ct* mutations including *ct* lethals contained *mdg4* in the 7B region of the X chromosome².

Using *in situ* hybridization with polytene chromosomes, we have analysed the localization of different mobile elements in the X chromosome of the ct^{MR2} strain (Figs 1–3). Larvae were examined at various times, in particular almost immediately after obtaining the ct^{MR2} strain and 2 years later. In all cases, the localization of mobile elements was exactly the same (the number of individual larvae analysed totalled 20). Thus, different mobile elements including *mdg* (*copia*-like elements), P-elements and FB-elements, have rather stable localizations. However, the analysis of novel substrains derived from ct^{MR2} , as a result of transposition event(s) in *cut* or other loci, reveals a dramatic change in the localization of mobile elements (Figs 1, 2). The two most extensively studied examples are shown in Fig. 1. The substrains analysed were ct^+ revertants which occurred simultaneously with unstable mutations at the *singed* or *white* loci. All mobile elements showed evidence of transposition, with their appearance at new, and disappearance from old, sites. In the case of $ct^{MR2} \rightarrow ct^+ sn^{MR2}$, 20 simultaneous transposition events were counted, and 12 were found for the $ct^{MR2} \rightarrow w^{MR1} ct$ mutation.

It is important to stress that all individuals of the new derivative strains possess the same distribution of mobile elements—this means that all transposition events occurred simultaneously

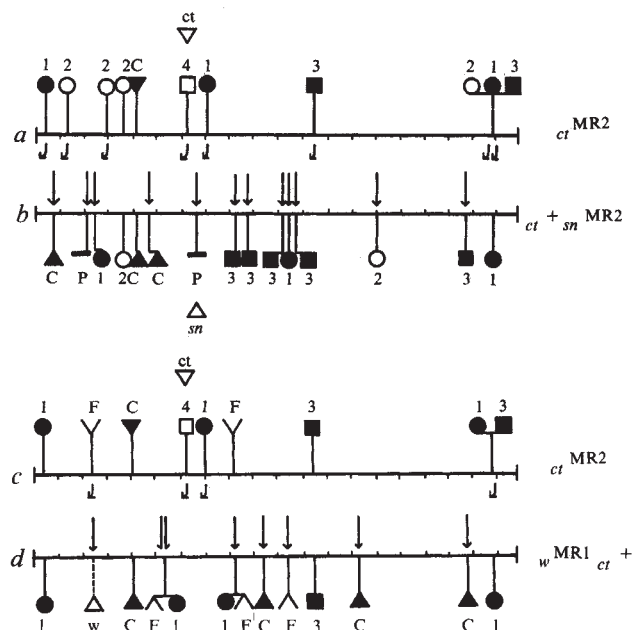


Fig. 1. Schematic presentation of the results obtained for *in situ* hybridization of ct^{MR2} , $ct^+ sn^{MR2}$ and $w^{MR1} ct^+$ X chromosomes with different probes. *a, c*, Distribution of hybridization sites along the ct^{MR2} X chromosome; *b*, that of the $ct^+ sn^{MR2}$ X chromosome; *d*, that of the $w^{MR1} ct^+$ X chromosome. In *a, b* the hybridization was performed with *mdg1* (1), *mdg2* (2), *mdg3* (3), *mdg4* (4), *copia* (C) and P-element (P); in *c, d* with *mdg1*, *mdg3*, *mdg4*, *copia*, P-element and FB-element (F). Triangles indicate the location of mutations characteristic of the strains analysed. Arrows show transposition events: the loss of mobile elements ($\downarrow$) and their insertion ($\uparrow$). Here and in the other figures, the 20 divisions on the line representing the X chromosome indicate the 20 major divisions of the chromosome on a Bridges map, the centromere being to the right. The strain ct^{MR2} remained unstable for 1.5 years, thereafter the transposition rate decreased, but later on increased. The strain was homozygous with respect to the X chromosome. ct^+ reversions and novel unstable mutations were observed in the males of the ct^{MR2} strain. To obtain the corresponding substrains, we used one of two methods. (1) The mutants were crossed with females bearing attached X chromosomes. Further, only the X chromosomes of male larvae were analysed. (2) The mutants were crossed with females possessing an X chromosome with a multiple inversion *FM4*, *y^{31d}*, *sc⁸ dm B* that excluded crossing-over. In this case, novel substrains homozygous with respect to the X chromosome (originating from ct^{MR2} X chromosome which has avoided crossing-over) were obtained. This made it possible to analyse both male and female X-chromosomes.

in the same germ cell. Had different transpositions occurred at different stages, there would be no homogeneity within the strains.

Figure 2 shows two other examples of multiple transpositions in the X chromosome of ct^{MR2} derivatives. In these cases, the ct^{MR2} mutation did not change, but a new unstable mutation occurred at the *garnet* (*g*) or *Bar* (*B*) locus. As the ct^{MR2} mutation was conserved, the *mdg4* remained in the 7B region. However, in both cases, several transpositions were found for other *mdg* elements and for FB-elements. Thus, the loss of *mdg4* from the 7B locus is not necessary for the induction of transposition. It should be pointed out that, in $ct^{MR2} g^{MR1}$, a *g* mutation seems to be induced by a *copia* insertion, as *g⁺* reversion always coincides (four cases were tested) with a loss of *copia* from the 12BC region.

We have also analysed several other ct^{MR2} derivatives for localization of *mdg1* or *copia* (Fig. 3). It is clear that almost always two to four transposition events occur in the X chromosome.

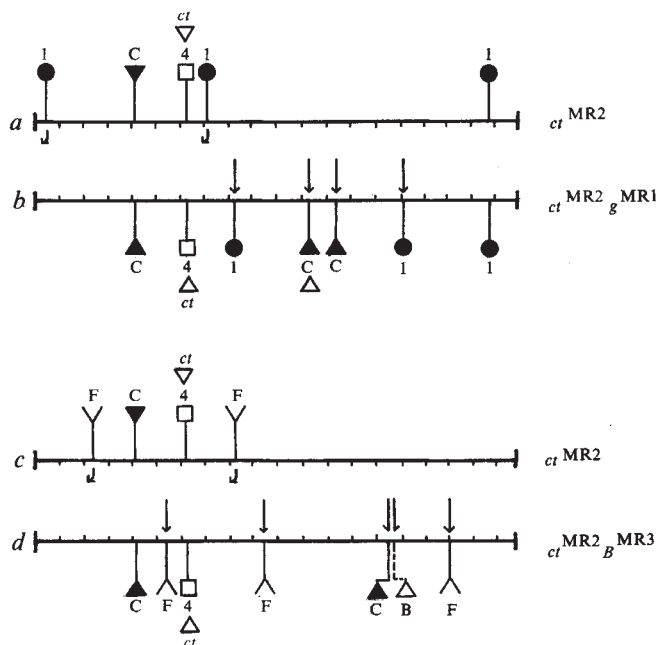


Fig. 2 Schematic presentation of the results obtained for *in situ* hybridization of ct^{MR2} , $ct^{MR2g^{MR1}}$ and $ct^{MR2B^{MR3}}$ X chromosomes with different probes. *a*, *c*, Distribution of hybridization sites along the ct^{MR2} X chromosome; *b*, that of the $ct^{MR2g^{MR1}}$ X chromosome; *d*, that of the $ct^{MR2B^{MR3}}$ X chromosome. In *a*, *b* the hybridization was performed with *mdg1*, *mdg4* and *copia*; in *c*, *d* with *mdg1*, *mdg4*, *copia* and FB-element. Designations are the same as in Fig. 1. The method used to isolate the substrain was as described in Fig. 1 legend.

The ct^{MR2} strain is not the only strain in which multiple transpositions can be observed in few germ cells. The ct^6g^2 strain (from the Mid-America *Drosophila* stock centre, Bowling Green State University), destabilized by a cross with the P-factor-containing strain *MRh12/Cy* (ref. 4), is another example. In the destabilized strain, a reversion to the g^+ and ct^+ type occurs at a rate of $\sim 10^{-3}$. In half of the cases, a double ct^+g^+ reversion takes place. The X chromosomes from stable and destabilized ct^6g^2 strains contain four *copia* sites (Fig. 4); different individuals possess the same distribution of *copia* sites. In the offspring of one ct^+g^+ revertant, however, one *copia* site was lost and two new sites were accepted (Fig. 4). Thus, three transposition events concerning only one *mdg* element occurred in the same germ cell.

We conclude, therefore, that in *Drosophila melanogaster* strains destabilized by hybrid dysgenesis, transpositions occur in only a few germ cells, but are numerous in these particular cells. 'Transposition bursts' have occurred in these cells.

Transposition bursts involve at least three major types of mobile elements: (1) mobile dispersed genes (*mdg*) or *copia*-like sequences^{5,6}; (2) foldback sequences⁷; and (3) P-elements⁸. Hence, all of these may be mobilized by a similar mechanism.

The total number of transposition events should be at least an order of magnitude greater than that determined in our experiments. We analysed the locations of only six different families of mobile elements and in only one chromosome. Even then, up to 20 simultaneous transposition events were detected; thus, the total number may be several hundreds.

Transposition bursts seem to occur only rarely in germ cells ($\sim 10^{-3}$) but this value may be an underestimate, as we expect that the insertion of a mobile element would occasionally be lethal and the respective germ cells or embryos would not survive. In fact, the homozygous ct^{MR2} strain is characterized by a high rate of appearance of sex-linked lethals¹.

The question arises as to which factors control multiple transposition. In our system (ct^{MR2}), P-factor seems to be an impor-

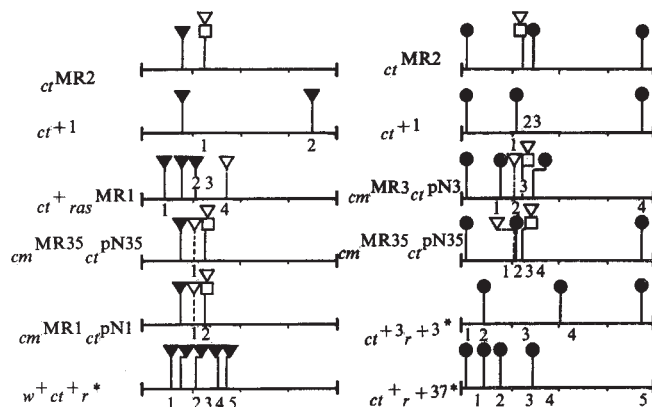


Fig. 3 Results of *in situ* hybridization of the X chromosomes from different ct^{MR2} derivatives with *mdg1* and *copia*. Most of the substrains appeared as the result of a mutation in ct^{MR2} . Those marked by asterisks were obtained from the $ct^{MR2r^{MR1}}$ substrain (which, in turn, originated from ct^{MR2}). The numbers represent the number of the transposition event. Designations are the same as in Fig. 1. Triangles indicate the locations of mutations in the individual strains.

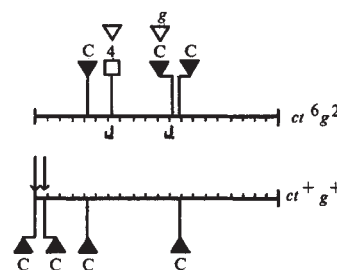


Fig. 4 Comparison of the distribution of *mdg4* and *copia* in the stable and destabilized ct^6g^2 strain and the ct^+g^+ double revertant obtained from ct^6g^2 . Designations are the same as in Fig. 1.

tant component^{2,3}. However, although the P-factor is present in all cells of a given organism, transpositions occur in only a few of them. Probably some other trigger mechanism exists.

The saltatory character of transposition may have a significant role in evolution. It is well known that the insertion of mobile elements is an important factor in spontaneous mutagenesis⁹. Therefore, transposition bursts should lead to simultaneous multiple mutagenesis, creating an organism that differs in many features from its parents. One of the problems in theories of evolution is how to explain the appearance of new complex properties requiring several independent genetic changes, each of which is not selectively advantageous—transposition bursts may provide the answer. Multiple mutations could occasionally create such complex features, which may then be retained by selective pressure.

In our system, transpositions occur at a premeiotic stage and, as a result, novel mutants sometimes appear in clusters¹. This may help changed genomes to survive in further generations.

Finally, transposition events can sometimes lead to gross chromosomal rearrangements. Thus transposition bursts may also assist in creating the reproductive isolation of a new substrain, the process having a key role in speciation.

We thank Drs D. Finnegan, G. Rubin and S. Potter for the clones containing *copia*, P-factor and FB-elements, Dr M. M. Green for the *MRh12/Cy* stock, and Dr M. Ashburner for critical reading of the manuscript.

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Control of tubulin gene expression in the parasitic protozoan *Leishmania enriettii*

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The life cycle of parasitic protozoa of the genus *Leishmania* consists of two morphologically distinct forms¹: (1) amastigotes, the form of the parasite that resides inside macrophages of the mammalian host, which are non-motile and possess only a residual flagellum; and (2) promastigotes, the extracellular forms that live in the gut of the sandfly vector, which are highly motile and possess a single prominent flagellum. During the developmental transformation of amastigotes to promastigotes, the biosynthesis of α - and β -tubulin proteins is dramatically increased², presumably to provide sufficient tubulin for synthesis and maintenance of the flagellum. We show here that the level of α - and β -tubulin mRNA in *Leishmania enriettii* promastigotes is significantly greater than that in amastigotes, as determined by both Northern blot analysis and by *in vitro* translation of cellular RNA. These results show that the regulated expression of the tubulin genes is controlled at the level of mRNA accumulation in *L. enriettii*, by contrast with *Leishmania mexicana*, in which the control of gene expression has been reported to be at the level of translation.

The α - and β -tubulins are the major developmentally regulated gene products during transformation of *Leishmania* amastigotes to promastigotes. The α - and β -tubulin mRNAs are the most abundant messages present in the promastigote stage of the life cycle³. Biosynthesis of α - and β -tubulin occurs at a low level in amastigotes but is dramatically increased during their transformation to promastigotes². On the basis of *in vitro* translation experiments, Wallach *et al.*³ have suggested that control of tubulin biosynthesis may occur at the level of translation. In a recent extension of their work on *L. mexicana*, Fong *et al.*¹⁸ have used heterologous tubulin probes and have found similar levels of tubulin mRNA in promastigotes and amastigotes. In contrast, we find, using homologous cloned copies^{4,5} of the α - and β -tubulin genes, significantly more α - and β -tubulin mRNA in promastigotes than in amastigotes of *L. enriettii*.

L. enriettii promastigotes and amastigotes were grown as described in Fig. 1 legend and total nucleic acid extracted. Equal amounts of total nucleic acid from promastigotes and amastigotes were resolved on denaturing agarose gels, transferred to nitrocellulose and probed sequentially with the α - and β -tubulin genomic clones. The filters were re-probed with a cloned ribosomal gene as an internal control. Figure 1 and Table 1 show that the promastigotes contain a minimum of 4–5-fold more α - and β -tubulin mRNA than the amastigotes. Similar results were obtained when the amount of α - and β -tubulin mRNA was measured by *in vitro* translation in the rabbit reticulocyte system (see Table 1). Based on these results, we conclude that the control of α - and β -tubulin synthesis occurs primarily at the level of mRNA accumulation.

The control of tubulin gene expression has been studied in several other systems and has, in general, been found to occur

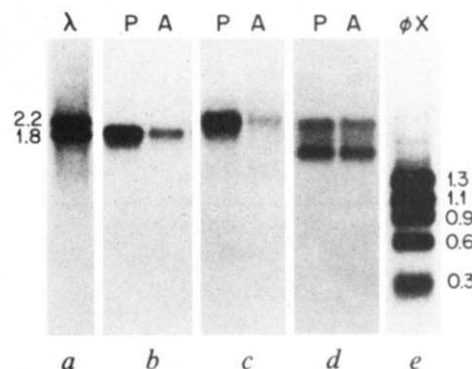


Fig. 1 Estimation of α - and β -tubulin RNA levels in promastigotes and amastigotes by Northern blot analysis. Five micrograms of total nucleic acid isolated by phenol–chloroform extraction from promastigotes (P) or amastigotes (A) of *Leishmania enriettii* were separated on a 1% agarose–formaldehyde gel¹² and blotted onto nitrocellulose. The same filter was sequentially hybridized⁴ with nick-translated¹⁵ α -tubulin clone pLTI (ref. 5) (b), β -tubulin clone pLE β 3 (S.M.L. and D.F.W., in preparation) (c) and with a ribosomal DNA clone (d) from *Plasmodium lophurae*¹⁶. Lane a contains *Hind*III fragments of λ DNA and lane e contains *Hae*III fragments of Φ X174 DNA hybridized to homologous probes. The numbers indicate the size of these molecular weight markers in kilobase pairs. The results in lanes b and c indicate that the α - and β -tubulin mRNAs in *L. enriettii* are both ~2 kilobases long. In addition, a heavier exposure of the autoradiogram in c (not shown) reveals lightly hybridizing bands both above and below the main band.

Methods: Promastigotes were grown to a density of $\sim 2 \times 10^7$ organisms ml^{-1} in Schneider's medium containing 10% heat-inactivated fetal calf serum. Amastigotes were grown by subcutaneous injection of either promastigotes or amastigotes into the noses of male Hartley guinea pigs. Lesions were allowed to develop for about 5 weeks, and parasites were isolated and purified by Percoll gradient centrifugation as described elsewhere¹⁷. This method yielded viable amastigotes at ~95% purity. RNA was prepared by suspending about 10^{10} parasites in 10 ml of 50 mM HEPES pH 7.5, 50 mM KCl, 10 mM EDTA, then lysing the cells with 0.1% Sarkosyl, and immediately extracting three times with 2 vol. phenol–chloroform and once with 2 vol. chloroform.

Table 1 Quantitation of tubulin RNA in promastigotes and amastigotes

	Promastigote/amastigote ratio
α-Tubulin	
Northern blots	
Expt 1	3.5:1
Expt 2	3.9:1
<i>In vitro</i> translation	1.9:1
β-Tubulin	
Northern blots	
Expt 1	5.3:1
Expt 2	4.0:1
<i>In vitro</i> translation	3.7:1

Autoradiograms of the Northern blots in Fig. 1 were quantitated by densitometry. Duplicate lanes were run for each determination, the integrated areas under the peaks were averaged, and these averaged areas were then normalized using the area under the ribosomal RNA peaks. This normalization adjusts for any small differences in the amount of total RNA actually loaded onto promastigote or amastigote lanes. The amount of tubulin RNA measured for the amastigote lane was then arbitrarily assigned a value of 1.0, and the relative amount for promastigotes was calculated. Experiment 1 was performed with RNA isolated by phenol–chloroform extraction (see Fig. 1), while expt 2 was performed with RNA isolated by the guanidinium isothiocyanate procedure¹². For *in vitro* translation, 2 μg of the RNA samples used in Fig. 1 were translated in the rabbit reticulocyte lysate system¹³ (25 μl total volume), and the products were resolved on an 8.5% Laemmli gel¹⁴. The α - and β -tubulin translation products were quantitated by densitometry of the autoradiogram.

at the level of mRNA accumulation⁶⁻¹¹. Specifically, flagellar regeneration in *Chlamydomonas reinhardtii* is accompanied by an increase in α - and β -tubulin mRNA levels as determined by *in vitro* translation and Northern blot analysis^{6,7}. We find that this is also the case for the α - and β -tubulin genes of *L. enriettii*. This is in contrast to the *L. mexicana* system^{3,18} in which similar amounts of tubulin mRNA are reported to be present in the two forms of the parasite. Our results, based both on the direct measurement of mRNA levels by Northern blot analysis using homologous probes and on the *in vitro* translation of mRNA in the rabbit reticulocyte system, clearly indicate that there is several-fold more α - and β -tubulin mRNA in *L. enriettii* promastigotes than in amastigotes. There are two possible explanations for the differences found in the two *Leishmania* systems: either the control of tubulin levels is different in the two species, or the results reflect a difference between the heterologous probes used in the *L. mexicana* experiments¹⁸ and the homologous probes used in our experiments. Thus, the stage-specific regulation of tubulin expression in *L. enriettii* is controlled at the level of mRNA accumulation. Whether this control is exerted through regulation of transcription or through RNA processing or degradation remains to be determined.

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Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide

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Table 1 Natriuretic effect of synthetic auriculin in intact rats

Dose (μg per kg)	$\Delta \dot{V}$ ($\mu\text{l min}^{-1}$)	$\Delta U_{\text{Na}} \dot{V}$ ($\mu\text{equiv min}^{-1}$)	$\Delta U_{\text{K}} \dot{V}$ ($\mu\text{equiv min}^{-1}$)
1.2 (4)	25.5 $\pm$ 9.7	2.5 $\pm$ 1.1	1.6 $\pm$ 0.2
2.4 (4)	41.3 $\pm$ 19.5	6.7 $\pm$ 4.1	4.4 $\pm$ 1.4
5.0 (4)	52.8 $\pm$ 6.5	9.1 $\pm$ 1.0	3.7 $\pm$ 0.5
7.2 (3)	112.0 $\pm$ 12.8	18.3 $\pm$ 0.5	3.1 $\pm$ 0.8

Synthetic peptide was administered as a bolus injection to rats (average weight 399 g; number of animals in parentheses) anaesthetized with Inactin (100 mg per kg) and maintained on a constant infusion of normal saline (2.2 ml h⁻¹). The change in each parameter was assessed by the difference between the average of three control periods (10 min each) and the first experimental period (maximum response). Data are expressed as the mean $\pm$ s.e. $\dot{V}$, urine flow rate; $U_{\text{Na}} \dot{V}$, urinary sodium excretion rate; $U_{\text{K}} \dot{V}$, urinary potassium excretion rate. Control values for the 15 animals were: $\dot{V}$, 10.3 $\pm$ 2.9 $\mu\text{l min}^{-1}$; $U_{\text{Na}} \dot{V}$, 0.93 $\pm$ 0.5 $\mu\text{equiv min}^{-1}$; and $U_{\text{K}} \dot{V}$, 1.6 $\pm$ 0.4 $\mu\text{equiv min}^{-1}$.

chromatography on a CN reverse-phase column, which resulted in the association of vasorelaxant activity with a single UV-absorbing peak (Fig. 1d).

Automated microsequence analysis of the purified substance revealed that it was a 24-amino acid residue peptide rich in glycine, serine and arginine (Fig. 2). This peptide is smaller than the ANFs purified to apparent homogeneity by deBold and Flynn¹² and by Grammer *et al.*¹³. It also differs from the sequences reported recently by Currie *et al.*¹⁴ in that it has an additional arginyl residue at the amino terminus.

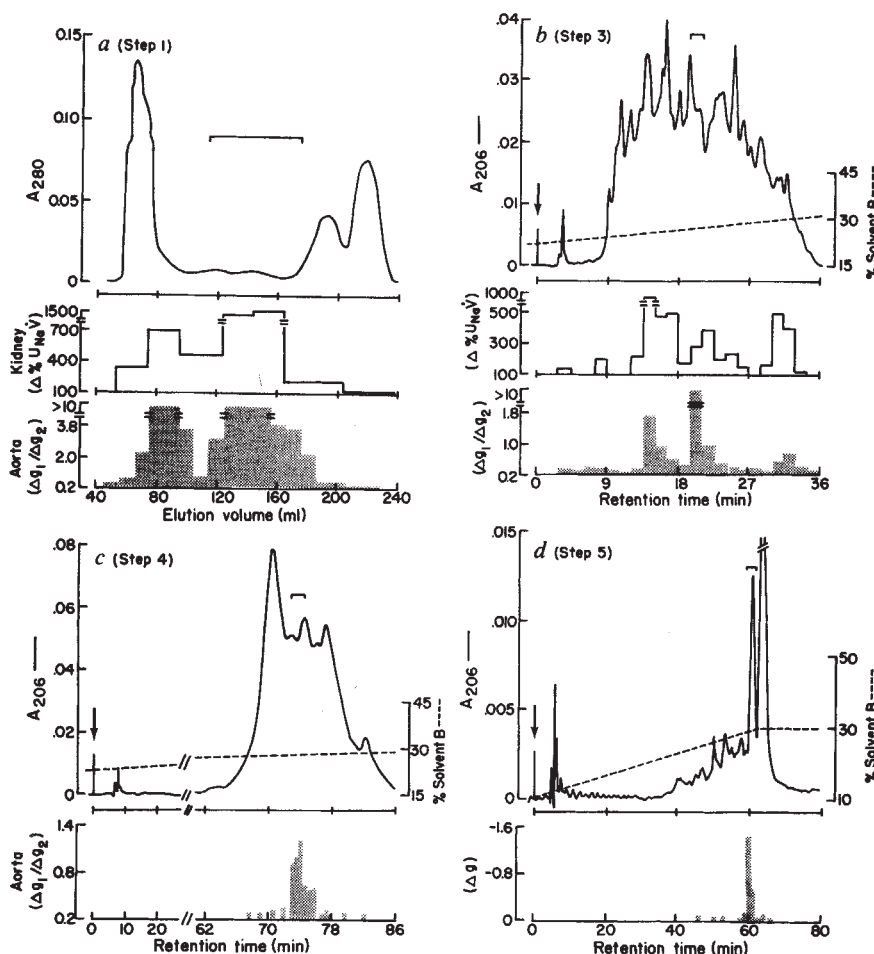
Solid-phase synthesis of the peptide shown in Fig. 2 has been accomplished, with a yield of over 150 mg (see Fig. 3 legend for methods). The synthetic peptide had vasorelaxant activity paralleling that of partially purified natriuretic factor (Fig. 3). Its activity was enhanced by allowing it to oxidize slowly, and the activity of the fully oxidized form was very similar to that of the purified peptide (Fig. 3), suggesting the Cys 4-Cys 20 disulphide configuration proposed in Fig. 2.

The synthetic peptide was natriuretic both in the intact rat (Table 1) and in the isolated rat kidney (Table 2). In the latter, perfused in the absence of vasoconstrictors, the peptide increased renal vascular resistance, filtration fraction and glomerular filtration rate (Table 2). In contrast, in the isolated kidney pre-contracted with endogenously generated angiotensin, the peptide decreased vascular resistance (not shown). These effects are similar to those previously described for partially purified peptide, confirming our conclusion that the ANF can have renal vasoconstrictive or vasorelaxant effects

Mammalian atria contain potent natriuretic and diuretic substances^{1,2} which exist in high- and low-molecular-weight forms^{2,3} and which appear to be associated with atrium-specific granules⁴. The natriuretic effect of atrial extract is largely accountable for by its renal haemodynamic effects⁵⁻⁷; atrial extracts also antagonize hormone- and non-hormone-induced contraction of the isolated rabbit aorta⁸⁻¹⁰ and isolated rat kidney vasculature⁶. We have completely purified a low-molecular-weight natriuretic and vasoactive substance from rat atria and characterized it as a 24-amino acid peptide. Synthetic peptide, produced by solid-phase synthesis, mimics biological effects of crude atrial extract and purified peptide; its activity is enhanced by slow oxidation, suggesting a disulphide (Cys 4-Cys 20) configuration for the native peptide. If secreted into blood, this atrial natriuretic peptide ('auriculin B') could be a novel peptide hormone of considerable importance to renal and cardiovascular homeostasis.

Atrial natriuretic factor (ANF) was purified from an acetic acid extract of atria from 1,400 male Wistar rats by gel filtration and reverse-phase HPLC (Fig. 1). Fractions were assayed for natriuretic activity either in the intact rat¹ or in isolated perfused rat kidney^{6,11}, and for vasorelaxant activity in rabbit thoracic aortic rings¹⁰ contracted with either angiotensin II or histamine. Two broad regions of natriuretic and vasorelaxant activities were found on Sephadex G-50, corresponding to molecular weights of >15,000 and <6,000 (Fig. 1a). Application of the low-molecular-weight material to a C₁₈ column resulted in a broad active fraction which, upon re-chromatography, was resolved into three peaks of coincident vasorelaxant and natriuretic activity (Fig. 1b). One of these was further purified by re-application to the same column (Fig. 1c) followed by

Fig. 1 Purification of natriuretic and vasorelaxant factors from rat atria. Right and left atria from 200 male Wistar rats were homogenized in 8 vols of 1 M acetic acid containing 1 mM phenylmethylsulphonyl fluoride, 3 mM EDTA and 5 μ M pepstatin A, centrifuged (10,800g for 30 min), and the pellet rehomogenized in 4 vols of buffer. The pooled supernatant was neutralized with ammonium hydroxide¹, centrifuged and lyophilized. *a*, Step 1: gel filtration on a 2.5 $\times$ 45 cm column of Sephadex G-50 (fine) equilibrated with 1 M acetic acid. The extract was reconstituted with 6 ml buffer, centrifuged and applied to the column; flow rate was 0.63 ml min⁻¹ and 15.9-min fractions were collected. Aliquots of each fraction were dried (Savant Speed-Vac concentrator), reconstituted in phosphate-buffered saline and assayed for natriuretic activity (0.3 ml) in the intact rat¹ and for vasorelaxant activity (50 μ l) in rabbit aortic rings¹⁰. Rings were first contracted with 2.9×10^{-8} M Ile⁵-angiotensin II, then washed, allowed to return to baseline tension and contracted a second time with 1.5×10^{-8} M angiotensin II in the presence of unknown sample; results are expressed as the ratio of the contractile force (Δg tension) generated by the first and second contractions ($\Delta g_1/\Delta g_2$). Six additional extracts were purified similarly and the lyophilized low-molecular-weight region (horizontal bracket) from each was reconstituted with 0.1% aqueous trifluoroacetic acid (TFA), pooled and centrifuged. Step 2 (not shown): one-sixth of the pooled material (representing 1,400 rat hearts), adjusted to 15% acetonitrile (CH₃CN), was applied to a 0.39 $\times$ 30 cm μ Bondapak C₁₈ column, using a Waters U6K injector and solvent delivery system, and eluted at a rate of 0.1 ml min⁻¹ with a linear gradient of solvents A (0.1% TFA) to B (CH₃CN) from 85:15 to 45:55 over 40 min. Aliquots of fractions were assayed for natriuresis in the isolated kidney^{6,11} and for vasorelaxant activity as described above. A broad region of coincident natriuretic and vasorelaxant activity eluted between 16 and 20 min. The active fractions from six successive runs were pooled and dried overnight. *b*, Step 3: re-chromatography of the step 2 material on the C₁₈ column. The sample was reconstituted in solvents A/B 78:22, and chromatographed (in 12 separate applications) at 1.0 ml min⁻¹ using a gradient of 22–34% B over 48 min. Aliquots of fractions were tested for natriuretic (100 μ l) and vasorelaxant (30 μ l) activities as described for step 2. Fractions from each of the three active peaks were pooled and dried overnight. The combined second peak (bracket) from the 12 runs was purified further. *c*, Step 4: re-chromatography of the second peak from step 3. The sample was reconstituted in A/B 77:23, applied to the C₁₈ column and eluted at 0.4 ml min⁻¹ using a gradient of 23–29% B over 90 min. The bracket indicates fractions with vasorelaxant activity that were pooled from each of six applications. *d*, Step 5: HPLC of the step 4 material on a 0.39 $\times$ 30 cm μ Bondapak CN column. The solvent system used was A = 0.1% TFA in H₂O and B = 0.055% TFA in CH₃CN. The sample was reconstituted in A/B 90:10, and chromatographed (in three separate applications) at 0.6 ml min⁻¹ using a gradient of 10–30% B over 60 min. Vasorelaxant activity was determined by the reduction in tension (Δg) produced in histamine-contracted aortic rings (see Fig. 3 legend for methods). A portion of the active peak (bracket) was re-chromatographed (not shown), giving a single detectable UV peak (retention time 60 min); the remainder was used for sequencing.



depending on the presence of vasoconstrictors⁶. The renal vasoconstrictive effect seems to be exerted preferentially in the efferent arteriole, since it leads to an increase in glomerular filtration rate and in the filtered load of sodium. This latter effect can account for the natriuresis observed in the isolated kidney (Table 2). The vasorelaxant effect predominates in the pre-contracted isolated kidney and possibly in intact animals, leading to an increase in renal blood flow^{7,15} and a shift of flow to the inner medulla¹⁵, phenomena known to induce natriuresis. Thus, the available data support the view that the natriuretic effect can be explained by renal haemodynamic actions⁶. Whether this peptide has, in addition, a direct tubular action remains to be demonstrated.

The synthetic peptide, like partially purified preparations, also has potent relaxant effects on the rabbit aorta pre-contracted with histamine (Fig. 3), noradrenaline^{8–10}, angiotensin II¹⁰ and K⁺-depolarization¹⁰ (not shown). No vasoconstrictive effect was detected in aortic rings. The ability to antagonize vasoconstrictors might explain the lowering of blood pressure by atrial extracts¹. Although no consistent effect on blood pressure was observed in assay rats, preliminary studies in anaesthetized dogs show that constant infusion of the synthetic peptide (at 0.1 μ g per kg per min) causes a 10–15% fall in mean arterial pressure.

We have reported here the first synthesis of a natriuretic and vasoactive factor present in mammalian atria. It would be premature to conclude that the structure proposed (Fig. 2) represents the complete sequence of the naturally occurring peptide. The large-molecular-weight active substance in atrial extracts

Table 2 Effects of synthetic auricularin on renal function in isolated perfused rat kidney

	Control	Experimental
GFR (ml min ⁻¹)	0.43 $\pm$ 0.05	0.63 $\pm$ 0.03*
FF	0.014 $\pm$ 0.002	0.021 $\pm$ 0.001*
RVR (mm Hg ml ⁻¹ min)	2.9 $\pm$ 0.1	3.9 $\pm$ 0.3*
$\dot{V}$ (μ l min ⁻¹)	19.8 $\pm$ 4.8	97.6 $\pm$ 19.4*
FL _{Na} (μ equiv min ⁻¹)	60.2 $\pm$ 7.9	90.1 $\pm$ 5.2*
T _{Na} (μ equiv min ⁻¹)	60.0 $\pm$ 7.6	84.2 $\pm$ 4.8*
U _{Na} $\dot{V}$ (μ equiv min ⁻¹)	0.66 $\pm$ 0.35	6.01 $\pm$ 1.99*
FE _{Na} (%)	0.97 $\pm$ 0.38	6.6 $\pm$ 2.0*
U _K $\dot{V}$ (μ equiv min ⁻¹)	0.44 $\pm$ 0.19	1.46 $\pm$ 0.16*
FE _K (%)	19.8 $\pm$ 5.9	52.1 $\pm$ 6.2*

Functioning isolated rat kidneys were perfused in a closed-circuit system with 60 ml of oxygenated perfusate (Krebs–Henseleit bicarbonate buffer, 7.5 g per dl albumin, glucose and amino acids) at an effective perfusion pressure of 90–100 mm Hg (refs 6, 11). After control clearance periods, 150 ng of synthetic peptide were added to the perfusate. Effects on each parameter were noted immediately and reached a maximum during the third 10-min clearance period; these peak values are given as the experimental data. GFR, glomerular filtration rate; FF, filtration fraction; RVR, renal vascular resistance; $\dot{V}$, urine flow rate; FL_{Na}, filtered load of sodium; T_{Na}, tubular reabsorption of sodium; U_{Na} $\dot{V}$, urinary sodium excretion rate; FE_{Na}, fractional sodium excretion; U_K $\dot{V}$, urinary potassium excretion rate; FE_K, fractional potassium excretion. Results are the mean $\pm$ s.e. of four kidneys.

*P < 0.05 compared with control (Student's *t*-test).



Fig. 1 Synthesis and use of oligonucleotide probes. The sequence of amino acids 4–12 of rat auricularin⁹ is shown along with the sequence of the four oligonucleotide mixtures, a and b for region 1, and c and d for region 2. R = A or G, Y = T or C, N = A, G, T or C. Each oligonucleotide mixture was synthesized on a Biosearch SAM I oligonucleotide synthesizer by a modification of the standard phosphotriester method using mesitylenesulphonyl chloride in the presence of *N*-methylimidazole as condensing reagents^{28,29} and purified by polyacrylamide gel electrophoresis. Double-stranded cDNA was synthesized from rat atrial poly(A)⁺ RNA. After the sequential addition of *Eco*RI and *Sal*I oligonucleotide linkers¹³ the cDNA was ligated to the plasmid expression vector pUC9 (ref. 14) and transformed onto *Escherichia coli* MC1061 (ref. 30). 5 µg of rat atrial poly(A)⁺ RNA yielded about 25 ng of cDNA, size-selected to greater than 300 base pairs (bp), and gave a library of about 200,000 independent recombinants. These were plated on Millipore Triton-free nitrocellulose filters, replica-plated and the library was stored frozen on glycerol-impregnated filters at -70 °C (refs 18, 19). Replica filters of the library were processed for hybridization^{15–17}. After baking, the filters were washed overnight at 68 °C with shaking in a large volume of 3×SSC (20×SSC = 3 M NaCl, 0.3 M sodium citrate pH 7.5), 0.1% SDS and then prehybridized in 6×SSC, 0.1% SDS, 1 mM EDTA, 5× Denhardt's solution³¹, 0.05% sodium pyrophosphate at 50 °C for at least 2 h (ref. 16). The filters were then hybridized with 2.5×10⁶ c.p.m. (1 pmol ml⁻¹) ³²P-labelled oligonucleotide mixture per filter, in hybridization solution containing 100 µg ml⁻¹ tRNA at 45 °C for 1 h in a shaking water bath. The thermostat was then lowered to 25 °C and the bath allowed to equilibrate overnight. The filters were washed twice in 6×SSC, 0.1% SDS at room temperature for 15 min, then washed at 35 °C (for probes c and d) or 39 °C (for probes a and b) for 1–2 min. The final washing temperature was obtained from the empirical formula of Suggs *et al.*³², $T_d = 4(G + C) + 2(A + T)$. The filters were then dried and autoradiographed with intensifying screens.

		4	5	6	7	8	9	10	11	12	13	
Amino acids		Cys	Phe	Gly	Gly	Arg	Ile	Asp	Arg	Ile	Gly	
Possible codons	5'	TGY	TTY	GGN	GGN	CGN	ATY	GAY	CGN	ATY	GGN	3'
						AGR	ATA		AGR	ATA		
probe a	3'	ACR	AAR	CCN	CCN	GC	5'					
probe b	3'	ACR	AAR	CCN	CCN	TC	5'					
probe c						3'	TA ^R _T	CTR	CGN	TA ^R _T	CC	5'
probe d						3'	TA ^R _T	CTR	TCN	TA ^R _T	CC	5'

a

GGCTGAGAGAGAAACAGAGAGTGGCCGAGACAGCAACATCAGATCTGCCCCGACCCACGCCAGC

100

ATG GGC TCC TTC TCC ATC ACC AAG GGC TTC TTC CTC TTC CTG GCC TTT TGG CTC CCA GGC CAT ATT GGA GCA
Met Gly Ser Phe Ser Ile Thr Lys Gly Phe Phe Leu Ala Phe Trp Leu Pro Gly His Ile Gly Ala

150

AAT CCC GTA TAC AGT GCG GTG TCC AAC ACA GAT CTG ATG GAT TTC AAG AAC CTG CTA GAC CAC CTG GAG GAG
Asn Pro Val Tyr Ser Ala Val Ser Asn Thr Asp Leu Met Asp Phe Lys Asn Leu Leu Asp His Leu Glu Glu

200

250

AAG ATG CCG GTA GAA GAT GAG GTC ATG CCT CCG CAG GCC CTG AGC GAG CAG ACC GAT GAA GCG GGG GCG GCA
Lys Met Pro Val Glu Asp Glu Val Met Pro Pro Gln Ala Leu Ser Glu Gln Thr Asp Glu Ala Gly Ala Ala

300

CTT AGC TCC CTC TCT GAG GTG CTT CCC TGG ACT GGG GAA GTC AAC CCG TCT CAG AGA GAT GGA GGT GCT CTC
Leu Ser Ser Leu Ser Glu Val Pro Pro Trp Thr Gly Glu Val Asn Pro Ser Gln Arg Asp Gly Gly Ala Leu

350

400

GGG CGC GGC CCC TGG GAC CCC TCC GAT AGA TCT GCC CTC TTG AAA AGC AAA CTG AGG GCT CTC CTC GCT GGC
Gly Arg Gly Pro Trp Asp Pro Ser Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu Arg Ala Leu Leu Ala Gly

450

500

CCT CCG AGC CTG CGA AGC TCA AGC TGC GGC GGT AGG ATT GAC AGG ATT GGA GGC CAG AGC GGA CTA GGC
Pro Arg Ser Leu Arg Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Gly Ser Gly Leu Gly

550

TGC AAC AGC TTC CCG TAC CGA AGA TAA CAGCCAAATCTGCTCGAGCAGATCGCAAAGATCCCAAGCCTTGCGGTGTCTACACAG
Cys Asn Ser Phe Arg Tyr Arg Arg TAA

600

650

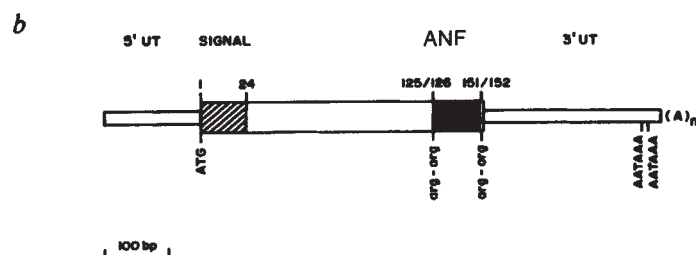
CTTGGTCGATTCGACATGAGAGTGGTGAATACCCCTCTGAGCTGCGAGCTCTCTCTCTCATCTATCATCATGATGATTAAGTGTAGATGAT

700

750

GGTTTAGTGAGGCTTACCTCTCCACTCTGCATATTAAAGGTAGATCTCACCCCTTTTCAGAAAGCAGTTGGAAAAAATAAATCCCAATAAAT

TCAGCACCACGGAC (A)_n



c

122 130 140 150 152

ArgSerLeuArgArgSerSerCysPheGlyGlyArgIleAspArgIleGlyAlaGlnSerGlyLeuGlyCysAsnSerPheArgTyrArgArgOH

(ref. 6)

(ref. 10)

(ref. 7)

(ref. 7)

(ref. 9)

Fig. 2 a, Nucleotide sequence of precursor ANF. The nucleotide sequence was established by the chain-termination method³³ using cDNA fragments subcloned into the M13 vectors mp8 and mp9 (ref. 34). All sequences were determined on both strands with the exceptions of nucleotides 755–790. The sequence for this region was obtained from another cDNA clone isolated using pNF1 as a hybridization probe. The basic Arg-Arg dipeptides, which are implicated in the processing of precursor ANF, are boxed, while the two AATAAA sequences involved in polyadenylation are underlined. **b**, Schematic representation of the structure of precursor ANF. The precursor sequence, amino acids 1–152, is shown with the putative signal peptide marked by cross-hatching and the auricularin sequence marked by stippling. The positions of the two Arg-Arg dipeptides (amino acids 125–126 and 151–152), which are potential processing sites, are shown. The 5'- and 3'-untranslated regions (UT) are marked and the two putative polyadenylation signals, AATAAA, are identified. **c**, Amino acid sequences of rat and human atrial peptides. The sequences of other biologically active peptides^{6,7,9,10} are shown. The Ile at position 134 (marked with an asterisk) is a Met in the human sequence¹⁰.

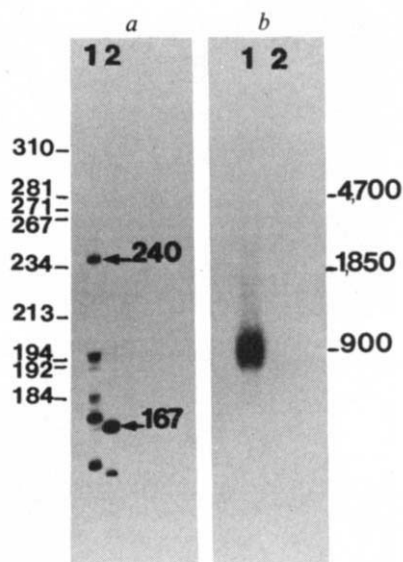


Fig. 3 Size determination of precursor ANF mRNA. *a*, Primer extension. Five μ g pNF1 DNA were digested with *Bgl*II, treated with bacterial alkaline phosphatase and 5'-end-labelled with [γ - 32 P]ATP by polynucleotide kinase. The kinase was heat-inactivated, the DNA digested with *Dde*I and the 167-bp *Dde*I-*Bgl*II fragment (nucleotides 3–170), 32 P-labelled at the *Bgl*II end only, was recovered from a polyacrylamide gel. One-fifth of the eluted DNA fragment was co-precipitated with 1 μ g rat atrial poly(A)⁺ RNA^{35,36}, resuspended in 40 μ l 80% formamide, 0.4 M NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA (refs 37, 38), heated to 68 °C for 10 min and hybridized at 55 °C for 3 h. Following ethanol precipitation the annealed primer-template complex was incubated with unlabelled deoxynucleoside triphosphates and reverse transcriptase and the size of the primer extension products estimated by electrophoresis on a 5% acrylamide, 7 M urea gel. Lane 1, primer-extension products; lane 2, unextended primer fragment. Sizes of marker fragments are given in base pairs. The full-length primer extension product (240 nucleotides) is 73 nucleotides longer than the primer fragment. Since the *Dde*I priming site is 3 nucleotides from the end of pNF1, the cDNA clone is about 70 nucleotides short of being full length. A strong intermediate band of about 195 nucleotides is also seen and may represent a region of secondary structure in the mRNA. *b*, Northern blot analysis. Poly(A)⁺ RNA (2 μ g) isolated from rat atrium, or rat ventricle, was fractionated by electrophoresis on a 1.4% agarose gel containing methyl mercuric hydroxide³⁹. The RNA was transferred to a nitrocellulose filter⁴⁰ and probed with nick-translated pNF1 DNA. Lane 1, atrial poly(A)⁺ RNA; lane 2, ventricular poly(A)⁺ RNA. The positions of 28S and 18S rRNA markers are given in bases.

The sequences of atrial natriuretic peptides reported to date differ slightly in length at both the N- and C-termini, which may be a reflection of variable proteolytic processing of a precursor. We used these amino acid sequences to design oligonucleotide probes to screen a rat atrial cDNA library cloned in the vector pUC9 (refs 13, 14) (Fig. 1). These probes were synthesized to correspond to two non-overlapping regions of the peptide (Fig. 1). Due to the degeneracy of the codons, we chose to synthesize two oligonucleotide pools for each region. Thus, region 1 was covered by two tetradecamer oligonucleotide pools, a and b, each consisting of 64 sequences, while region 2 was covered by another two tetradecamer pools, c and d, each consisting of 72 sequences.

The pools of oligonucleotides were used to screen the atrial cDNA library by colony hybridization^{15–17}. Four replica filters were prepared from each master filter^{18,19}, and each replica was probed with one of the oligonucleotide pools. A colony was considered positive if it hybridized with one of the region 1 and one of the region 2 probes. One clone was picked as the most likely candidate for encoding auriculin as it not only hybridized

very strongly to the oligonucleotide probes, but also represented an atrium-specific sequence. This was demonstrated by its hybridization to a random primed atrial, but not ventricular, cDNA probe.

Nucleotide sequence analysis confirmed that this clone (pNF1) encodes an auriculin precursor. When the atrial cDNA library was re-screened with this cDNA insert, approximately 0.5% of the colonies hybridized, showing that ANF is a major species in rat atrial mRNA. The complete nucleotide sequence and structure of the precursor is shown in Fig. 2. A single open reading frame encoding a 152 amino acid sequence extends from an ATG initiation codon at nucleotide 69 to a TAA termination codon at nucleotide 525. Within this reading frame the sequence of the various forms of biologically active atrial natriuretic peptides can be identified (Fig. 2c). The sequence in Fig. 2a has a 5'-untranslated region of 68 nucleotides and a 3'-untranslated region of 266 nucleotides. Two potential polyadenylation signals, AATAAA (ref. 20), are seen in the 3'-untranslated region. Multiple polyadenylation sites have also been observed in other genes^{21,22}.

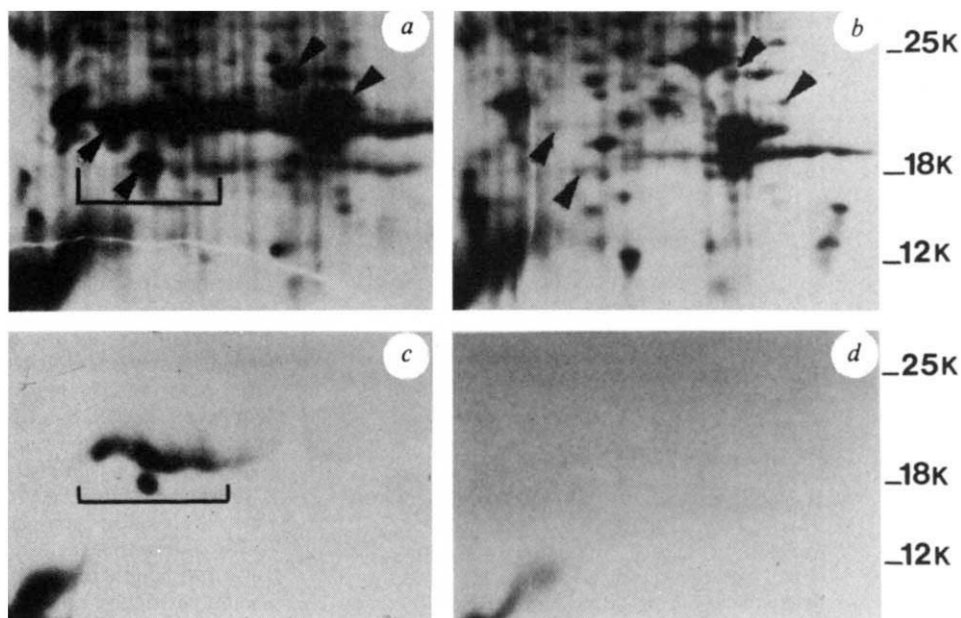
To confirm that the cDNA clone, pNF1, represents an essentially full-length transcript of precursor ANF mRNA, we isolated a restriction enzyme fragment from pNF1 and used it to prime cDNA synthesis from atrial poly(A)⁺ RNA. The results (Fig. 3a) show that pNF1 is about 70 nucleotides short of a full-length cDNA clone. The mRNA for precursor ANF is therefore about 850 nucleotides long (excluding the poly (A) tail). This conclusion is supported by the results of a Northern blot (Fig. 3b), in which pNF1 hybridizes to an atrial mRNA of approximately 900 nucleotides but is not detected with ventricular mRNA (Fig. 3b).

Other evidence supports our identification of the reading frame of the ANF precursor. The precursor has been proposed to be a secretory protein, found in atrial granules, and would therefore be expected to have a signal peptide, and indeed, the sequence following the proposed initiation codon (Fig. 2a) encodes a stretch of amino acids, starting at position 9 which, though unusually rich in phenylalanine, has the hydrophobic character of a signal peptide²³. Cleavage of this sequence could occur after either the glycine at position 23 or the alanine at position 24 (the preferred amino acids at the signal peptide cleavage site), producing a signal peptide of normal length. Eukaryotic genes do not have a ribosome-binding site sequence like that seen in prokaryotes, although a consensus sequence, CC^ACCATGG, is found around the initiation codon²⁴. In the case of precursor ANF, this sequence is CCAGCATGG. Finally, the identification of the reading frame is supported by the fact that the cDNA clone is similar in size to the mRNA, and that the molecular weight of the precursor protein as predicted from the sequence matches closely with the observed size (see below). A schematic version of precursor ANF is shown in Fig. 2b.

The diversity of previously identified amino acid sequences encoding natriuretic peptides (see Fig. 2c) does not allow precise determination of the sites at which precursor ANF is processed. However, the sequence of auriculin is flanked on both sides by basic dipeptides (Arg-Arg at positions 125–126 and 151–152) which have been identified in other hormone precursors as proteolytic processing sites²⁵. The varying lengths of the active peptides identified by the different groups could result either from the precursor being processed at sites other than Arg-Arg or from an artefactual proteolytic cleavage of the precursor during purification. The Arg-Arg dipeptide at the C terminus of auriculin is immediately followed by the TAA termination codon and, as such, is similar to that of the corticotropin-releasing factor precursor²⁶.

The predicted molecular weight (MW) of precursor ANF is 16,720. To determine the actual precursor size, atrial mRNA encoding precursor ANF was purified by hybrid selection²⁷, using pNF1 DNA, and translated *in vitro*. The selected translation products were then analysed by two-dimensional gel electrophoresis and compared with the total translation products from atrial and ventricular mRNA (Fig. 4). Translation of the

Fig. 4 Two-dimensional gel fractionation of cell-free translation products encoded by poly(A)⁺ RNA isolated from: *a*, rat atria; *b*, ventricle, or selected by hybridization²⁷ of atrial (*c*) or ventricular (*d*) poly(A)⁺ RNA to pNF1 DNA. 5 µg of plasmid pNF1 DNA were immobilized on 1-cm² nitrocellulose disks and hybridized with 5 µg of poly(A)⁺ RNA for 3 h at 50 °C in 20 mM PIPES, pH 6.4, 1 mM EDTA, 65% formamide, 5 × SSC, 0.1% SDS. Filters were then washed extensively in 10 mM Tris-Cl pH 7.5, 0.15 M NaCl, 1 mM EDTA, 0.1% SDS at 70 °C and at 37 °C in the same buffer without SDS. Hybridized RNA was eluted in H₂O at 100 °C in the presence of 50 µg tRNA for 1 min, quick-frozen at -70 °C, thawed and precipitated by the addition of 2 vols absolute ethanol. Hybrid-selected RNA and total poly(A)⁺ RNA was translated using a commercially available rabbit reticulocyte lysate system in the presence of 250 µCi ml⁻¹ ³⁵S-methionine. Acid-precipitable radioactivity (1 × 10⁶ c.p.m.) translated from total atrial and ventricular poly(A)⁺ RNA or 1 × 10⁵ and 5 × 10⁴ c.p.m. of acid-precipitable radioactivity translated from hybrid-selected atrial and ventricular RNA, respectively, was fractionated by two-dimensional gel electrophoresis. The first dimension consisted of isoelectric focusing using a pH 3.5–10 gradient⁴¹ while the second dimension was a 15% acrylamide-SDS gel⁴². The gels were equilibrated in 1 M sodium salicylate, dried and fluorographed at -70 °C for 24 h. The electrophoretic positions of several atrium-specific translation products in the 12,000–30,000 MW range are marked by arrows. The translation products encoded by pNF1-hybrid-selected atrial RNA are indicated by brackets.



hybrid-selected RNA yielded at least three proteins with molecular weights of 16,500–18,500 (Fig. 4c). These translation products selected by pNF1 represent abundant atrial species which are not present in the ventricle.

The function of the N-terminal portion of the precursor is not clear. Higher-molecular-weight forms of biologically active material have been isolated^{4,7,9–11} but they have less potent natriuretic and diuretic effects than auricularin. These activities could result from the proteolytic cleavage of the precursor in the assay system releasing auricularin. The N-terminal fragment lacking auricularin has not been identified *in vivo*, and therefore has no biological activity ascribed to it. Knowledge of the sequence of the entire precursor will allow us to express the complete ANF precursor, or defined fragments of it, in yeast or bacteria, and to investigate their biological activities.

Note added in proof: We have sequenced the primer extension product and confirmed the sequence from nucleotides 7 to 54. Nucleotides 1–6 are, however, incorrect, and probably result from a cloning artefact.

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Structure of rat atrial natriuretic factor precursor deduced from cDNA sequence

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The atrium of the heart contains peptides, termed atrial natriuretic factors (ANFs), diuretic¹ and smooth-muscle-relaxing^{2–4} activities. In view of its potent effects on salt metabolism in the kidney and on vascular smooth muscle, ANF is considered to play an important part in the control of fluid volume and vascular function. Several different ANF peptides varying in size have been isolated^{3–10} and their amino acid sequences determined^{4,6–10}. Analysis of the

sequences of the peptides suggests that they are derived by proteolysis from the same precursor. To examine this hypothesis, we have cloned cDNAs of the ANF precursor using rat atrial mRNA, determined its nucleotide sequence and deduced its amino acid sequence. The ANF precursor consists of 152 amino acid residues including a putative signal peptide sequence. This sequence contains the amino acid sequences of all the ANF peptides reported to date^{4,6-10}.

For use as hybridization probes, two pentapeptide segments of rat ANF peptide⁴ which gave the least numbers of possible combinations of codons were chosen for constructing 14-mer mixed oligonucleotides designated oligo I and oligo II (Fig. 1a). The ability of the synthetic oligonucleotides to specifically hybridize with mRNA for ANF was tested, using oligo I as a primer, by reverse transcription of poly(A) RNA isolated from rat atrium (Fig. 1b). Poly(A) RNA from ventricle was used as a control. Following urea-polyacrylamide gel electrophoresis, only one major cDNA band corresponding to 460 nucleotides in length was produced from atrial mRNA, but not from ventricular mRNA, suggesting specific hybridization of oligo I to ANF mRNA.

A library of rat atrial cDNA clones was constructed using the cloning vector of Okayama and Berg¹¹ (see Fig. 2 legend for

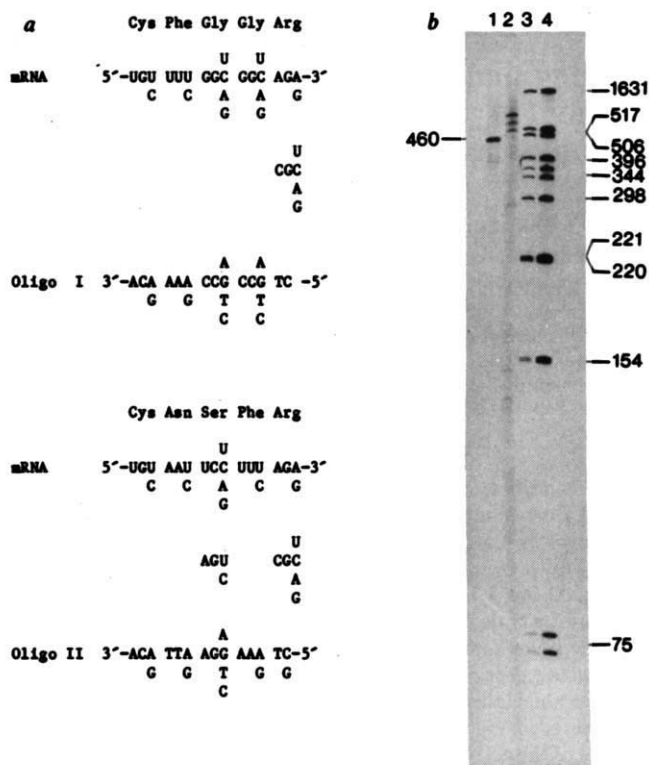


Fig. 1 *a*, Synthetic oligonucleotides used for screening cDNA clones for rat ANF. Mixed 14-mer oligodeoxynucleotides, oligo I and oligo II (custom-synthesized by American Bionuclear) are complementary to all possible coding sequences corresponding to the two pentapeptide sequences which had been found in ANF peptides⁴. Codons for Arg in oligo I and for Ser in oligo II were selected on the basis of the frequency of codon usage in rat mRNA. *b*, Electrophoretic analysis of cDNA products synthesized from poly(A) RNA using oligo I as a primer. Poly(A) RNA was isolated from atria and ventricles of Sprague-Dawley rats by the guanidine isothiocyanate-CsCl procedure²² followed by oligo(dT)-cellulose chromatography. cDNA was synthesized from 20 µg of poly(A) RNA obtained from atria (lane 1) or ventricles (lane 2), using ³²P-labelled oligo I (0.125 µg, 1.5 × 10⁸ c.p.m. µg⁻¹) as a primer for reverse transcriptase reaction, and was electrophoresed on an 8% polyacrylamide gel containing 7 M urea²³. *Hinf*I digests of pBR322 (NEN) were labelled with ³²P and used as markers for the sizes and radioactivity of synthesized cDNAs (lane 3, 1,200 c.p.m.; lane 4, 6,000 c.p.m.).

experimental details). By screening with oligo I, 56 hybridization-positive clones were selected from 900 transformants. Using oligo II as the second probe, candidates were narrowed down to eight clones. All eight clones had cDNA inserts of indistinguishable sizes with common restriction endonuclease sites for *Pst*I, *Xho*I, *Hinc*II and *Bgl*II.

The nucleotide sequence analysis of one of these clones, pOB8, by the method of Sanger *et al.*¹²⁻¹⁴ revealed that it lacks a 5'-noncoding sequence as well as the ATG initiation triplet.

A clone with a longer cDNA insert, pGH33, was isolated from a second atrial cDNA library prepared by the method of Gubler and Hoffman¹⁵ (see Fig. 2 legend). Its nucleotide sequence, determined by the methods of Sanger *et al.*¹²⁻¹⁴ and of Maxam and Gilbert¹⁶, was in agreement with that of pOB8 in the overlapping region. The complete nucleotide sequence of the ANF cDNA clones consisting of 786 residues is presented in Fig. 3.

The nucleotide sequence from residue 376 to residue 450 corresponds exactly to the previously determined amino acid sequence of ANF peptide (the boxed region in Fig. 3); it allowed us to define the reading frame for the entire ANF precursor and, thus, deduce its amino acid sequence. The distance from the 5' end to the position of oligo I was 461 nucleotides long, which agrees with the observed size (460 nucleotides) of the major cDNA product of the primer-extended reverse transcript of atrial mRNA (Fig. 1b). Therefore, the first ATG triplet (nucleotide residues 1-3) proximal to the 5' end of the sense strand was designated as the initiation codon. An unusually

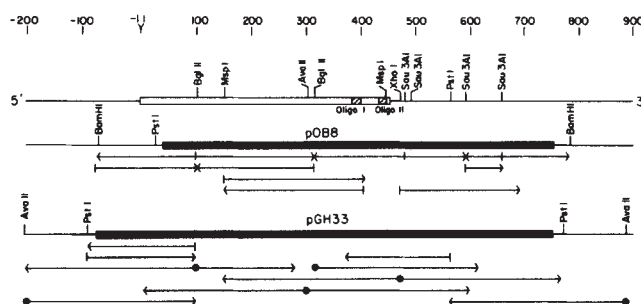


Fig. 2 Sequencing strategy of ANF cDNA. Clones pOB8 and pGH33 were isolated from two cDNA libraries prepared independently by the procedures of Okayama and Berg^{11,24} (method A) and Gubler and Hoffman¹⁵ (method B) respectively.

Methods: A: cDNA-plasmid recombinant molecules were prepared using 4 µg of poly(A) RNA from rat atria, 2.2 µg of dT-tailed pcD vector¹¹ and dG-tailed linker. *Escherichia coli* N-38, a substrain of HB101, was used for transformation and ampicillin-resistant transformants were screened by colony hybridization²⁵ at 37 °C or 41 °C with oligo I (Fig. 1a) labelled with ³²P at the 5' end. The hybridization-positive clones were re-screened with oligo II (Fig. 1a) at a hybridization temperature of 31 °C or 37 °C. B: The first cDNA strand was synthesized using 10 µg of poly(A) RNA as template which had been denatured by 10 mM of methylmercuric hydroxide²⁶. The RNA was replaced by the second cDNA strand using a mixture of RNase H, DNA polymerase I and DNA ligase. The double-stranded cDNA thus obtained was tailed with dCTP, annealed to *Pst*I-cleaved and dG-tailed pBR322 and introduced into *E. coli* N-38. Tetracycline-resistant transformants were screened by colony hybridization with ³²P-labelled *Msp*I fragment of pOB8 cDNA as a probe. The nucleotide sequences of the plasmid inserts pOB8 and pGH33 (solid boxes) were determined both by the dideoxy terminator method of Sanger *et al.*¹² (sequencing direction indicated by arrows with a short vertical line) and by the method of Maxam and Gilbert¹⁶ (indicated by arrows with solid circles). In the former, restriction fragments were subcloned into M13mp8 or M13mp9 (ref. 13) and Klenow fragment of DNA polymerase I or reverse transcriptase (ref. 14) was used in the chain-termination reaction. The open box indicates the coding region flanked by an initiation codon and a termination codon. The hatched regions represent the segments corresponding to synthetic oligonucleotides (oligo I and oligo II) used as probes.

-53 AGAGAGAAACAGAGACTGAGCGAGACAGCAACATCAGATCTGTGCCCGACCCAGCCAGC -1

1 ATG GGC TCC TTC TCC ATC ACC AAG GGC TTC TTC CTC TTC GGC TTT TGG CTC CCA GGC 60
Met Gly Ser Phe Ser Ile Thr Lys Gly Phe Phe Leu Phe Leu Ala Phe Trp Leu Pro Gly
10 20

61 CAT ATT GGA GCA AAT CCC GTA TAC ACT GCG CTG TCG AAC ACA GAT CTG ATG GAT TTC AAG 120
His Ile Gly Ala Asn Pro Val Tyr Ser Ala Val Ser Asn Thr Asp Leu Met Asp Phe Lys
30 40

121 AAC CTG CTA GAC CAC CTG GAG GAG AAG ATG CCG CTA GAA GAT GAG CTC ATG CCT CCG CAG 180
Asn Leu Leu Asp His Leu Glu Glu Lys Met Pro Val Glu Asp Glu Val Met Pro Pro Gln
50 60

181 CCC CTG AGC GAG CAG ACC GAT GAA CCG CCG GCA CTT AGC TCC CTC TCT GAG CTG CCT 240
Ala Leu Ser Glu Gln Thr Asp Glu Ala Gly Ala Ala Leu Ser Ser Leu Ser Glu Val Pro
70 80

241 CCC TGG ATC GGT GAA GTC AAC CCG TCT CAG AGA GAT GGA GGT GCT CTC GGC CGC GGC CCC 300
Pro Trp Thr Thr Gly Val Asn Pro Gln Arg Asp Gly Gly Ala Leu Gly Arg Gly Pro
90 100

301 TGG GAC CCC TCC GAT AGA TCT GGC CTC TTG AAA AGC AAA CTG AGC GCT CTG CTC GCT GGC 360
Trp Asp Pro Ser Asp Arg Ser Ala Leu Leu Lys Ser Lys Leu Arg Ala Leu Ala Gly
110 120

361 CCT CGG AGC CTG CGA AGG TCA AGC TGC TTC GCG GGT AGG ATT GAC AGG ATT GGA GGC CAG 420
Pro Arg Ser Leu Arg Arg Ser Ser Cys Phe Gly Gly Arg Ile Asp Arg Ile Gly Ala Gln
130 140

421 AGC GGA CTA GGC TGC AAC AGC TTC GCG TAC CGA AGA TAA GAGCAAAATCTGCTGAGCAGATCGCA 486
Ser Gly Leu Gly Cys Asn Ser Phe Arg Tyr Arg Arg ***
150

487 AAAGATCCCAAGCCCTTGGGCTGTGTGTCACACAGCTGTGTCATGTCACCATGAGAGCTGGTGAATACCTCTGGAGCT 565

566 GCAGCTCTCTGCTTCTATCATCAGATCGATGTTAAGTGTAGATGAGTGGTTTACTGAGGCGTTACCTCTCCCACTCT 644

645 GCATATTAAAGTAGATCTCACCCCTTTCAGAAAGCAGTGGCAAAAAATAATCGGAATAATTCAGCACCACGGAC 723

Fig. 3 Nucleotide sequence of rat ANF cDNA. Nucleotides are numbered in the 5' and 3' direction beginning with the first residue of the ATG triplet encoding the translational initiation methionine, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The deduced amino acid sequence is shown below the nucleotide sequence. A potential cleavage site for the signal peptide is indicated by an arrow between residues 24 and 25. Potential poly(A) addition signals (AATAAA)²⁷ are underlined. The boxed region shows the amino acid sequence previously determined by automated Edman degradation of the 25-amino acid ANF peptide⁴.

long inverted repeat sequence (nucleotides 467–482) lies immediately downstream from the TAA stop codon.

The nucleotide sequence of rat ANF cDNA reveals that the ultimate precursor of ANF peptide comprises 152 amino acids with a calculated molecular weight of 16,556. The amino-terminal 24-amino acid sequence appears to be a signal peptide¹⁷ as it contains a region which is rich in hydrophobic amino acids with bulky side chains (positions 10–18) and is followed by a region containing amino acids having small side chains¹⁸. While this work was in progress, Thibault *et al.*¹⁹ reported the amino-terminal sequence of a rat high-molecular-weight ANF, which corresponds with the stretch from Glu 78 to Asp 135 in the precursor sequence deduced in the present study.

The finding of the carboxy-terminal sequence -Arg-Arg (151–152) is intriguing as no active ANF peptide isolated and sequenced to date contains this structure^{4,6–9}. The possibility cannot be excluded that the cleavage of this pair of arginine residues may be related to the activation of ANF.

The recent findings linking atrial deficiency of natriuretic activity with hereditary congestive heart failure in a strain of Syrian hamster²⁰ and with spontaneous hypertension in rat²¹ strongly indicate that ANF has an important role in cardiovascular regulation. cDNA for ANF obtained in the present study may be useful in elucidating regulation of ANF mRNA synthesis and its involvement in pathogenesis of such cardiovascular diseases. The deduced amino acid sequence of the precursor of ANF should also facilitate elucidation of the mechanism of synthesis, processing and secretion of ANF.

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Cloning and sequence analysis of cDNA encoding a precursor for human atrial natriuretic polypeptide

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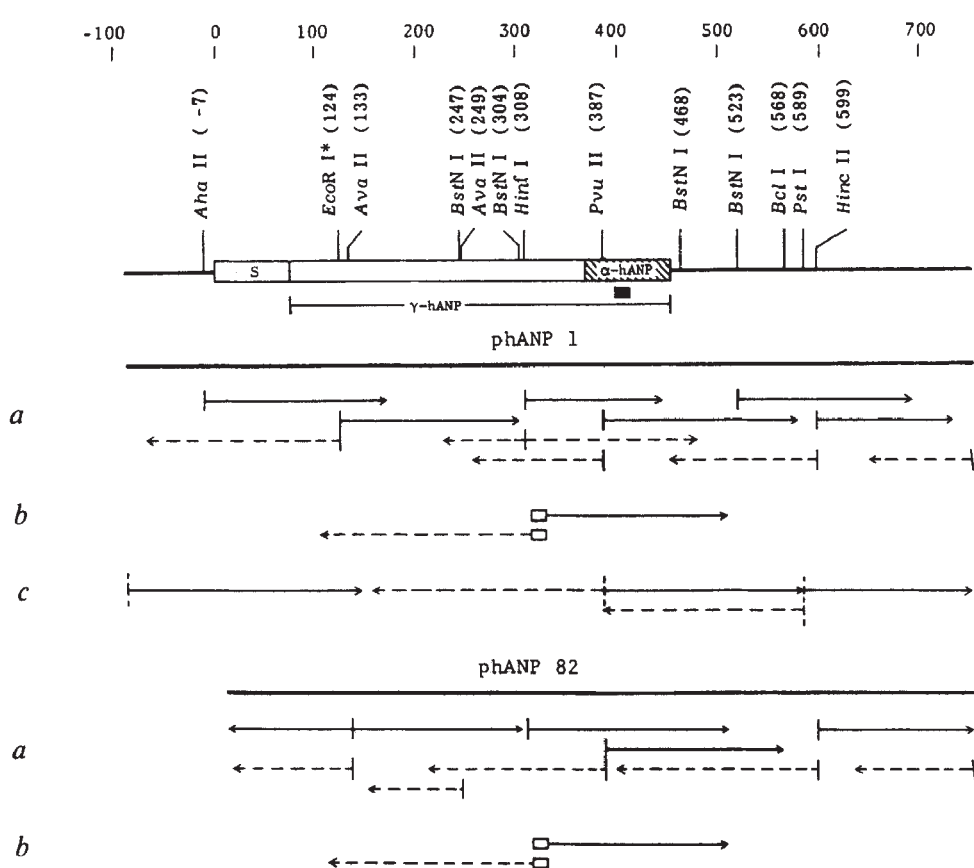
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Recent identification of natriuretic–diuretic activity in peptides isolated from human¹ and rat^{2–5} atrial tissue implicates them in the control of extracellular fluid volume and electrolytic homeostasis. The presence of multiple forms of the peptides ranging from 3,000 to 13,000 molecular weight (MW) suggests they may all derive from the same precursor^{1–6}. The established amino acid sequence of α -human atrial natriuretic polypeptide (α -hANP)¹, a 28-residue peptide with potent natriuretic activity, provided the means to elucidate the structure of the precursor for α -hANP and the gene encoding it. Here we report the cloning and sequence analysis of the cDNA of human atrial mRNA encoding a precursor of α -hANP. The cDNA encodes γ -human atrial natriuretic polypeptide (γ -hANP) of 13,000 MW, whose C-terminal 28 amino acid residues may be processed as α -hANP.

A library of cDNA clones from human atrial poly(A)⁺ RNA was constructed using the method of Okayama and Berg⁷. To probe cloned cDNA for the precursor of α -hANP, tetradecamer oligodeoxynucleotides representing all possible complementary sequences corresponding to amino acids 12–16, Met-Asp-Arg-Ile-Gly, of α -hANP (excluding the third nucleotide residue of the glycine codon) were synthesized by modified triester methods⁸ as two pools (oligo I and oligo II). The cDNA library was screened by hybridization with ³²P-labelled probes. Twenty-three clones, which hybridized with oligo II but not with oligo I, were isolated from about 40,000 transformants. Plasmids isolated from eight of these clones were submitted to nucleotide sequence analysis and all were found to carry a DNA segment encoding the N-terminal sequence (1–11) of α -hANP. Two plasmids of this group, phANP1, harbouring the longest

Fig. 1 Strategy of sequencing the cDNA inserts in clones phANP1 and phANP82. The restriction map displays only those restriction endonuclease sites relevant to the present work. They are identified by numbers indicating 5'-terminal nucleotides generated by cleavage. Nucleotides are numbered as in Fig. 2. S, Coding region for putative signal peptide; α -hANP; γ -hANP, region of the sequence of synthetic oligodeoxyribonucleotides used in probing cloned cDNAs. The direction and extent of sequence determinations are shown by arrows ($\rightarrow$, upper strand; $\leftarrow$, lower strand). The sites of 5'- and 3'-end-labelling are indicated by short vertical lines at the ends of arrows. Double slashes indicate that 5'-end-labelling was done on the sites located on the vector DNA. Dotted lines mean that the transcription primed by the general primers was started from sites located on the pUC8 plasmid¹⁸. Open boxes at the end of arrows indicate the region of the primer sequence detailed below.

Methods: The clones were obtained as follows. One mg of total RNA was extracted¹⁹ from atria of an 87-year-old female and a 61-year-old male post mortem. A total of 75 μ g of poly(A)⁺ RNA was isolated by oligo(dT)-cellulose binding procedure²⁰ using 10 mM Tris-HCl buffer, pH 7.2 containing 0.5 M LiCl₂, 10 mM EDTA and 0.5% SDS as a binding buffer. A cDNA library was constructed by the method of Okayama and Berg⁷ using 15 μ g of poly(A)⁺ RNA and 4.2 μ g of the vector-primer. *Escherichia coli* WA802 was used for transformation. Approximately 40,000 ampicillin-resistant transformants were obtained per μ g of starting mRNA and screened by hybridization²¹ at 38 °C for 16 h with the 5'-³²P-labelled mixtures of oligo I and oligo II. A second hybridization was done on the 85 positive clones with similarly labelled oligo I and oligo II at 40 °C and 38 °C for 16 h respectively. Plasmids were isolated from 8 clones out of 23 which hybridized with oligo II but not with oligo I. The chain-terminator nucleotide-sequencing method of Sanger *et al.*²² using oligo II as a primer showed that all 8 plasmids carried an insert which contained the coding sequence corresponding to the first 11 amino acids of α -hANP. Two plasmids, phANP1 and phANP82, having the longest inserts (about 950 bp and 850 bp respectively), were used for further sequencing by the following methods. *a*, Maxam-Gilbert method²³; *b*, method of Sanger *et al.*²², using as primers oligodeoxyribonucleotides of the sequences 5'-GATCGATCTGCCCTC-3' and 3'-CTAGCTAGACGGGAG-5' for lower and upper strands respectively; *c*, method of Sanger *et al.*²² after cloning three fragments of the insert generated by digestion with *Pst*I and *Pvu*II endonucleases into pUC8 plasmid¹⁸.



cDNA insert of about 950 base pairs (bp) and phANP82 (about 850 bp), were sequenced according to the strategy indicated in Fig. 1 legend.

Met Asp Arg Ile Gly
3'-TAC CT_A GC_G TAG CC-5' (oligo I)

3'-TAC CT_A TC_C TAG CC-5' (oligo II)

850 bp), were sequenced according to the strategy indicated in Fig. 1 legend.

Figure 2 depicts the complete nucleotide sequence of the cDNA insert of the phANP1 plasmid which is 843 bp long (excluding poly(A) sequence). All of the 3' portion of the mRNA upstream of the poly(A) tail⁹ was represented in the insert because about 80 consecutive A residues were found at the 3' end. Most of the 5' end portion of the mRNA also seems to be represented in the insert, because none of 58 independently isolated transformants had plasmids with an insert appreciably longer than that of phANP1 on cleared lysate analysis¹⁰.

The nucleotide sequence of phANP1 has a single large open reading frame, the first ATG codon appearing as 91-93 nucleotides from the 5' end (nucleotide residues 1-3 in Fig. 2). A TGA termination triplet is found 151 codons later. The 3'-untranslated region of 300 nucleotides contains the hexanucleotide sequence AATAAA (underlined in Fig. 2), which is known to precede polyadenylation sites in many eukaryotic mRNAs¹¹.

Figure 2 shows the amino acid sequence deduced from the 151 codons. The sequence of nucleotide residues 370-453, encoding amino acids 124-151, corresponds precisely to the amino acid sequence of α -hANP. In addition, residues 26-151 deduced from the base sequence 76-453 in the cDNA corresponds exactly to the natriuretic polypeptide of MW 13,000, designated γ -hANP, which has been recently identified in human atrial tissue. The complete sequence of 126 amino acids of γ -hANP has been established in our laboratory as starting from H-Asn-Pro-Met-Tyr-Asn- at its N-terminus and ending in -Asn-Ser-Phe-Arg-Tyr-OH at its C-terminus; α -hANP is the C-terminal 28 residues of γ -hANP (details to be published elsewhere).

In the cDNA sequence, the TAC codon for the C-terminal Tyr of γ -hANP is followed by the translational termination codon TGA. Therefore, the protein translated from the reading frame is apparently a precursor for both γ -hANP and α -hANP, and we have called the precursor pre-human atrial natriuretic polypeptide (pre-hANP).

The 25 amino acid residues preceding the γ -hANP sequence exhibit features characteristic of a signal peptide which is usually present at the N-terminal end of precursors of secreted proteins^{12,13}. The hydrophobic core¹⁴ corresponding to amino acids 9-20 includes 10 hydrophobic residues and is followed, 5 amino acids later, by an alanine residue at position 25, which very often occurs before the cleaving site of signal peptides¹⁴. The appreciable sequence similarity between the signal peptides of the precursor for α -hANP and human preproen-

Fig. 2 Nucleotide sequence of plasmid phANP1 cDNA insert and deduced amino acid sequence of the precursor for α -hANP. The nucleotide sequence of phANP1 and phANP82 cDNA inserts were determined as described in Fig. 1 legend. Both sequences coincided except for 99 5'-terminal nucleotides shown in the figure which were missing from the phANP82 insert. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of ATG triplet encoding the initiator methionine. The predicted amino acid sequence of the precursor for α -hANP is displayed above the nucleotide sequence, and its amino acid residues numbered beginning with the initiator methionine. Boxed sequence is of α -hANP. The arrow indicates the most likely processing site for signal peptidase. The hexanucleotide which precedes the site of polyadenylation in many eukaryotic mRNAs¹¹ is underlined.

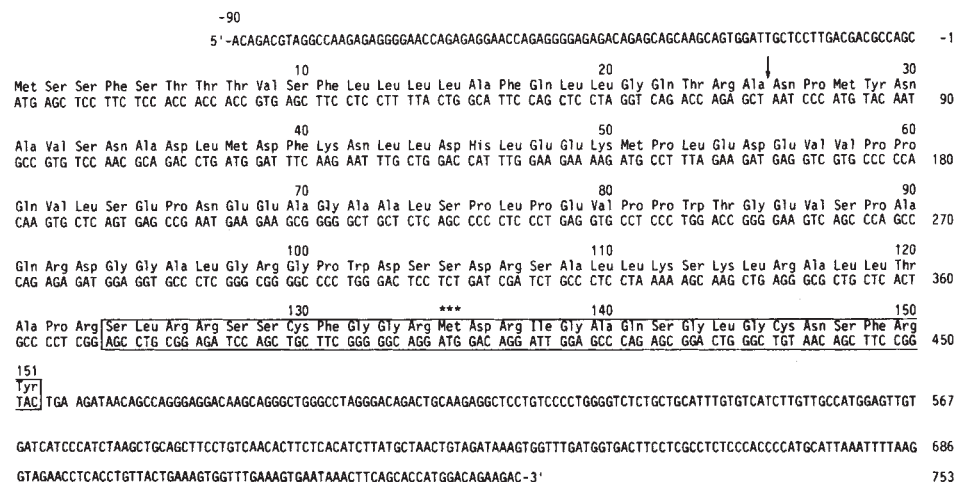
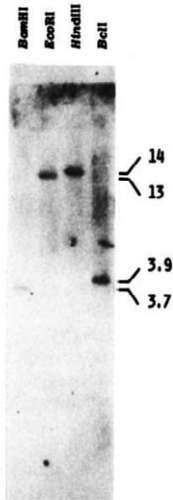


Fig. 3 Hybridization of human genomic DNA with a ³²P-labelled cDNA probe from plasmid phANP82. High-molecular-weight human DNA (8 µg) isolated from peripheral blood lymphocytes of several individuals by the procedure of Gross-Bellard *et al.*²⁴, using pronase instead of proteinase K, was digested to completion with 40 U each of various restriction endonucleases, electrophoresed on 1.0% agarose gel and blotted to a nitrocellulose filter²⁵. The ³²P-labelled DNA probe was prepared from a 608-bp *Pst*I fragment (nucleotides 10–588 plus 29 G residues extended from nucleotide 10 of Fig. 2) of the cDNA insert of phANP82 as described previously²⁶. 1.4 × 10⁷ c.p.m. of the probe were hybridized with the filter for 16 h at 65 °C and then washed as described²⁷. Numbers on the right of the figure indicate the size of the hybridized DNA fragment in kilobase pairs.



kephalin A¹⁵ (Met-Ala-Arg-Phe-Leu-Thr-Leu-Cys-Thr-Trp-Leu-Leu-Leu-Leu-Gly-Pro-Gly-Leu-Leu-Ala-Thr-Val-Arg-Ala, where indicated amino acid residues are underlined) was noted. The direct connection of γ -hANP to the putative signal

sequence suggests that removal of the signal peptide and disulphide bond formation may yield natriuretically active γ -hANP. Also, the location of α -hANP at the carboxyl end of γ -hANP suggests that further processing takes place after the sequence Pro-Arg (residues 122–123). Although the Pro-Arg sequence has not been regarded as a typical processing signal in the synthesis of many peptide hormones, gastrin-releasing peptide is known to be processed at a Pro-Arg sequence to yield its C-terminal decapeptide amide in the mammalian intestine¹⁶ and spinal cord¹⁷. α -hANP could be similarly processed out of the precursor.

The existence of multiple peptides with natriuretic activity in human atria led us to investigate (as described in Fig. 3 legend) whether the other related hANP genes are present in the human genome. Endonucleases *Bam*HI, *Eco*RI and *Hind*III do not have cleavage sites in the cDNA, but *Bcl*I has a cleavage site near the *Pst*I site (Fig. 1). The fragment to the right of the *Bcl*I site would not be expected to form a stable hybrid with the probe used because the complementary sequence region is only 21 nucleotides long. A single hybridizing DNA fragment can be seen in each lane of the gel (Fig. 3), which strongly suggests the existence of a single gene for hANP precursor in the human genome, although the presence of multiple genes comprising a repetition of the same sequence cannot be excluded.

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Astrophysical consequences of $n-\bar{n}$ oscillations

SIVARAM and KRISHAN¹ have suggested that the anomalously high flux of low-energy antiprotons observed in cosmic rays² is produced when free neutrons, which are possibly ejected in supernova explosions, undergo neutron-antineutron ($n-\bar{n}$) oscillations as expected in some grand and partially unified theories³. Such oscillations are strongly suppressed in an external magnetic field³⁻⁶ but by choosing the interstellar field strength, B , to be as low as 10^{-7} G and an oscillation time, $\tau_{n-\bar{n}} = 10^5$ s, these authors obtain¹, $\bar{n}/n \approx 10^{-4}$. Because $\bar{n}$ and n β -decay to $\bar{p}$ and p respectively, this implies $\bar{p}/p \leq 10^{-4}$, which compares with the observed value², $\bar{p}/p = (2.2 \pm 0.6) \times 10^{-4}$ at ~ 130 –370 MeV/n. (Contrary to their¹ remark, the ratio $\bar{p}/p$ cannot exceed the ratio $\bar{n}/n$, regardless of the number of neutrons ejected per proton in a supernova.)

However, before this suggestion, old limits on the stability of nuclear matter had been used to infer⁴⁻⁶ $\tau_{n-\bar{n}} > 10^6$ – 5×10^7 s. Also, direct observations of free neutron beams had yielded the limit⁷, $\tau_{n-\bar{n}} > 1.2 \times 10^5$ s, and this has been recently improved to⁸ $\tau_{n-\bar{n}} > 10^6$ s. Furthermore, the interstellar magnetic field strength must be $> 10^{-6}$ G to account for observations of pulsar signals⁹ and the galactic synchrotron background¹⁰. (We disagree that this value is "... given by equipartition arguments ... (with) ... no physical basis ...".) Thus, using these conservative limits, we obtain $\bar{n}/n \approx \bar{p}/p < 10^{-8}$ ($\tau_{n-\bar{n}}/10^6$ s)⁻² ($B/10^{-6}$ G)⁻². Moreover, this obtains at ~ 1 MeV/n, corresponding to the typical ejection velocity of $\sim 10^4$ km s⁻¹ in a supernova. Thus the observation that $\bar{p}/p \sim 10^{-4}$ at ~ 200 MeV/n cannot be accounted for by this mechanism.

Note that even in the total absence of a magnetic field, neutrons would sooner β -decay than oscillate into antineutrons. This sets an absolute upper limit $\bar{n}/n < 10^{-6}$ ($\tau_{n-\bar{n}}/10^6$ s)⁻², thus making this process uninteresting in any conceivable astrophysical or cosmological context.

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SIVARAM AND KRISHAN REPLY—We have not stated anywhere in our paper¹ that all of the 10^{57} neutrons ejected by a single supernova are at an energy ~ 200 MeV, in which case there would indeed be an excess of energy involved. The only claim made is that here is a possible mechanism for directly producing $\bar{p}$ at low (MeV) energies without any need for deceleration from energies of several GeV (with its attendant difficulties) inevitable in most models.

Second we felt that there was no need for Sarkar to have written down all the formulae for $n-\bar{n}$ transition probabilities. These are well known and are the same formulae that we used. In fact for a $B = 10^{-7}$ G and $\tau_{n-\bar{n}}$ of 10^5 s, he also obtains $\bar{n}/n \sim 10^{-4}$. Regarding his chief point of contention that the strength of the interstellar magnetic field must be $\sim 10^{-6}$ G; we are surprised that Sarkar has overlooked the fact that in the latter half of our paper we have pointed out that if this field is assumed it would considerably lower $\bar{p}/p$ ($< 10^{-6}$). (For 10^{-6} G, $\Gamma_B \gg \Gamma_{n-\bar{n}}$, and for 10^{-7} G, they are just equal.) We went on to point out that to build up the observed low-energy background of 10^{55} antiprotons in our Galaxy (as implied by the observed density of 10^{-4} eV cm⁻³) we would require a few thousand explosions which would occur in periods $\sim 10^6$ yr which is the same order as the diffusion time for the produced particles to spread over the Galaxy and produce the observed background. More generally one can say that the number of antiprotons produced is

$$\bar{p} = N \left(\frac{\Gamma_{n-\bar{n}}}{g u_n B} \right)^2 f t, \quad \Gamma_{n-\bar{n}} = \hbar / \tau_{n-\bar{n}}$$

where N is the number of neutrons per supernova, f the frequency of explosions and t the diffusion time $\sim 10^5$ yr. Thus if all low-energy antiprotons ($\bar{p} = 10^{55}$) in the Galaxy were produced by supernovae, then with $N = 10^{57}$, $f = 1/10$ yr, we would have an astrophysical constraint on the oscillation time $\tau_{n-\bar{n}}$. If $\tau_{n-\bar{n}} > 10^7$ s (as indirect evidence suggests), of course, the $\bar{p}$ production would be too low. Measurements of $\bar{p}$ should perhaps be made over a few MeV as this is the range where most supernova neutrons are produced.

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Formation of sapropels in the Tyro Basin

WE were most interested in the two companion papers pertaining to the recent formation of sapropels in the Tyro Basin, an isolated depression within the Hellenic Trench in the eastern Mediterranean Sea. Certainly the physiography within this trench system would be conducive to allowing localized near-bottom anoxia because of limited bottom water ventilation as related to outcrops of Messinian salt, as well pointed out by Jongsma *et al.*¹ and de Lange and ten Haven².

We are concerned, however, with their statement: "Assuming an undisturbed sedimentation and taking a relatively high sedimentation rate of 5 cm kyr⁻¹, we conclude that the recovered sediment column of 400 cm of solely sapropel to sapropelic sediment indicates a period of at least 80,000 yr."

Instead, we suggest that the 4-m long section was deposited in much less time. There is ample evidence of resedimentation events in similar isolated basins during the recent geological past and the present throughout the Hellenic Trench system³⁻⁵, as well as on the Mediterranean Ridge immediately south of the trench^{6,7}. In particular, redeposited sapropels have been identified in dated piston cores from these regions. Gravity-driven processes triggered by the active tectonism in the region have resulted in stratigraphically displaced and expanded-thickness sapropel sections^{8,9}, sometimes in excess of 4 m, and in dilution of organic matter content. As a result of this prevalent resedimentation process, cores from the trench show some of the highest sedimentation rates (to > 400 kyr⁻¹) yet measured in the Mediterranean Sea⁴. Thus, their conclusion of a 400-cm long sapropelic section representing 80,000 yr of undisturbed sedimentation is unrealistic. It is equally plausible that at least part of the sapropelic section cored in the Tyro Basin is redeposited, and equivalent to the upper sapropel (S_1) dated at $\sim 8,000$ yr BP and mapped over much of the eastern Mediterranean.

L-DGO contribution no. 3653.

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TROELSTRA *ET AL.* REPLY—We agree with McCoy and Stanley's view that the age at the bottom of the Tyro Basin core must be considerably younger than we had assumed in our papers, which were mainly based on shipboard observations. Shore-based analyses, still in progress, have produced different data. Because of the different bottom water regimes in the Tyro and Kretheus basins, we would like to consider each basin separately.

First, we want to stress that, at present, conditions suitable for sapropel formation, namely an anoxic and reducing environment due to halocline water stratification, do exist in the Tyro basin. Laboratory studies on core P46 indicate that sediments were not deposited in exclusively pelagic conditions. The most important argument for this is the presence of fining-upward sequences in the core, indicating transport by mass-flows. Furthermore, faunal and floral composition through the core is rather uniform and suggests a relatively warm climate. If the age at the bottom of the core were 80,000 yr, a fluctuating faunal and floral pattern would be expected, due to climatic changes. Moreover, the presence at the bottom of the core of well-preserved algae, one species still in possession of bright green-coloured chlorophyll, indicates that little or no diagenesis took place. This is contrary to what one would expect if the sediments were deposited at 80,000 yr BP.

In contrast to the Tyro Basin, bottom waters in the Kretheus Basin are well-ventilated at present. Core P45 consists of alternate dark-coloured (? resedimented sapropel) and lighter-coloured sediments. The latter contain abundant sand-sized grains of metamorphic rock indicating that sedimentation in the Kretheus Basin is also governed by mass-flow transport. Planktonic foraminiferal composition through the core is comparable with that in core P46.

We conclude that sedimentation rates in both basins must be high. At this stage, pending ^{14}C analyses, an entire Holocene age for both cores cannot be excluded. Resedimented S1 may be one of the major contributors to the sapropelic intervals in the cores, as correctly assumed by McCoy and Stanley.

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Sequence similarity among retroviruses—erratum*

RECENTLY Toh *et al.*¹ reported the existence of amino acid sequence similarity between the reverse transcriptase of certain retroviruses and the DNA polymerases of cauliflower mosaic virus (CaMV) as well as a region of hepatitis B virus (HBV) that probably encodes a DNA polymerase. For completeness, we draw attention to other regions of protein similarity between retroviruses (human T-cell leukaemia virus, HTLV; Moloney murine leukaemia virus, Mo-MuLV; and Rous sarcoma virus, RSV), HBV and CaMV that were not reported by Toh *et al.*

The sequences are presented in Table 1 and the relative positions of the similar regions indicated in Fig. 1. The common set of amino acids discussed by Toh *et al.* is conserved in the amino-terminal region of all retrovirus reverse transcriptases studied (regions I–III). These common sequences are located near the amino-terminus of the HBV protein and the centre of the CaMV polymerase. Another region of similarity, IV, not discussed by Toh *et al.*, is also found in all retroviruses and is present in the CaMV protein. Region V, reported by Toh *et al.*, to be present in Mo-MuLV and RSV, is also found on HTLV. We also draw attention to sets of amino acids that are conserved

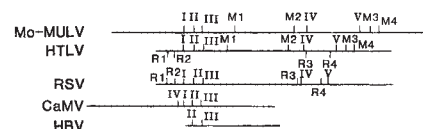


Fig. 1 Alignment of the polymerase gene products among five different viruses, depicting the regions shown in Table 1.

with respect to sequence and relative position between the reverse transcriptases of HTLV and Mo-MuLV (M1–M4) and HTLV and RSV (R1–R4).

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Table 1 Alignment of the similar regions in polymerase gene products among different viruses

REGION	VIRUS	SEQUENCE	REGION	VIRUS	SEQUENCE			
I	HTLV	114	D L R D A F F Q T P L	M3	HTLV	720	T D N G P A Y I S	
	Mo-MuLV	270	D L E D A F F C L R L		Mo-MuLV	974	T D N G P A E V S	
	RSV	107	D L E D C F F S I P L	M4	HTLV	745	H V P Y N P I T S S G L V E R S N	
	CaMV	355	D C E S G F W V L L		Mo-MuLV	799	H C A Y R P Q S S G Q V E R M N	
II	HTLV	150	H K V L P Q G F K N S F T L F	R1	HTLV	37	L Q A L Q H L V R K A L E A G H I E P	
	Mo-MuLV	305	H T R L P Q G F K N S F T L F		RSV	31	L V A L T Q L V E K E L S G H I E P	
	RSV	143	H K V L P Q G F K N S F T L F	R2	HTLV	61	N N P V F P V K K A N G T W R F I H D L R A T N	
	CaMV	384	H N V Y P F G L K Q A P S I F		RSV	55	N T P V F V I R K A S G S E R L E H D L R A V N	
	HBV	25	E R K I P M G V G L S P F L L	R3	HTLV	572	H V R S H	
III	HTLV	184	I L Q Y M D D I L L A S P S H		RSV	546	H V R S H	
	Mo-MuLV	338	I L Q Y V D D L L L A T S E	R4	HTLV	660	I W Q G D I T	
	RSV	177	M L H Y M D D L L L A S S H		RSV	633	I W Q T D F T	
	CaMV	413	C C V Y D D I L Y F S N N E					
	HBV	57	L A F S Y M D D V V L G A K S N					
IV	CaMV	331	D A Y N L P N K D E L L T L I R G K K I F S S F	R4	HTLV	660	I W Q G D I T	
	Mo-MuLV	727	E G K E I K N K D E I L A L K A L F L P K R L S		RSV	633	I W Q T D F T	
	HTLV	541	L A L G T F G R S S Q A P F Q A L P R L L S					
	RSV	556	F F T E G N E V A D S Q A T F Q A Y P L R E A K					
V	HTLV	677	L H V W V D T F S G A I S A					
	Mo-MuLV	930	L L V E I D T F S G W I E A					
	RSV	650	L A V I V D T A S S A I V V					
M1	HTLV	263	Q A L L					
	Mo-MuLV	469	Q A L L					
M2	HTLV	499	P P H K S A Q R A E L L G L					
	Mo-MuLV	673	P A G T S A Q R A E L I A L					

Molony murine leukaemia virus, Mo-MuLV²; human T-cell leukaemia virus, HTLV³; Rous sarcoma virus, RSV⁴; cauliflower mosaic virus, CaMV⁵; and hepatitis B virus, HBV⁶. Common amino acids are boxed and conservative substitutions with respect to HTLV are underlined. For each sequence, the first amino acid is numbered.

* This paper was published in the issue of 17 May without title and Fig. 1 due to printing errors.

The welfare stake

L.G. Goodwin

Of Mice, Models, and Men: A Critical Evaluation of Animal Research.

By Andrew N. Rowan.

State University of New York Press: 1984. Pp.323. Hbk \$34.50; pbk \$12.95.

Man and Mouse: Animals in Medical Research.

By William Paton.

Oxford University Press: 1984. Pp.192. Hbk £9.95; pbk £2.95.

AS Andrew Rowan says on p.31 of his book, "research on animals touches a very raw nerve in some people". Those who protest do so from diverse motives — they may identify with the helplessness of the animal or argue against "speciesism" as against sexism, racism and slavery. Some fight research they consider to be wasteful and frivolous, while others fear scientific progress of any kind. In recent years activist movements (reflecting the growth of violence in other spheres) have organized angry, destructive demonstrations; research animals are "liberated" and

terrorist groups break into laboratories and use letter bombs to intimidate those who dare to remind the public of the advances in curative medicine and in public health that have followed from experiments on living animals [Sir Andrew Huxley in the foreword to *Man and Mouse*].

Yet, at the same time, there is a more intelligent, growing public concern about the way in which animals are used in laboratories. Although there is need for debate, biomedical scientists have sometimes been unwilling to talk.

The appearance of two excellent, outspoken books, one from each side of the Atlantic, is well timed. Andrew Rowan from Tufts University School of Veterinary Medicine, has a clear appreciation of the value and necessity of animal experiments but for long has waged a campaign for animal welfare, for the introduction of effective United States Government legislation and for a reduction in the numbers of animals used in what he considers to be wasteful or unnecessarily painful experiments.

Rowan feels that "the old platitudes and the arrogant cliché that doctor knows best are no longer sufficient in the face of a more sophisticated approach by the animal welfare movement". He deals with the history of animal research in the United States and with the attitudes and assumptions it evoked, lists the numbers of animals used, attempts to assess the pain and suffering caused, and discusses the "rights" of animals. He thinks that too many animals are used needlessly in education at all levels and deplores the "science fairs" that lead to unnecessary suffering of animals at the hands of high-school students. He also criticizes the work of the primate centres, various aspects of behavioural, psychological and other

studies, and gives examples to support his belief that biomedical knowledge is being advanced at too great a cost in animal suffering. He believes that there is an opportunity for a great reduction in the wastage of animals in toxicological tests and for the revision of the requirements of the statutory bodies that demand them.

Sir William Paton, of the Department of Pharmacology in Oxford, has written a thoughtful, philosophical book that covers much of the same ground but from a different viewpoint. His objective is to make a strong case for *responsible* experimentation on live animals, and to look to the future. While there has been legislation in Britain on the conduct of animal experiments for more than 100 years, the Act of Parliament of 1876 is in course of revision and there is a European Convention on the use of animals in research.

In a reflective discussion of the ethical questions, Paton finds that the characteristic that gives human life a special value, different in quality from the lives of other animals, is the capacity to accumulate experience by the spoken, written and printed word and all that flows from it — the capacity to consent, to ask moral questions, to feel moral obligations and to feel responsible for animals. He finds no basis for claims that animals have "rights" (a human social concept linked with duties), but distinguishes between humans as moral *agents* and animals as moral *objects*, in respect of which humans accept duties. "Animal rights" may be replaced, with some gain, by the "moral worth of animal life".

Paton shows how, from the earliest times, accumulated knowledge from animal experiments (deliberate attempts to discover what was not yet known) has yielded great benefits for human and animal health. Many of these benefits were derived from work undertaken to acquire basic knowledge, with no particular benefit in view. Many problems still remain to be solved, especially in the control of diseases of impoverished, developing countries, and Paton warns that unreasonable harassment and restriction of the efforts to acquire new knowledge is likely to inhibit advance and cause unnecessary human and animal suffering. Of all forms of animal suffering — and the "natural" life of animals in the wild is full of stress and tragedy — only that incurred by experi-

ment, and the medical and veterinary practice based on it, offers the prospect of reducing further suffering and misery.

Both authors agree that the problem is to strike a balance and that Russell and Burch's "3 Rs" (put forward in *The Principles of Humane Experimental Technique*; Methuen, 1959) should be applied to all animals in experimental work — *replacement* by alternatives when possible, *reduction* in numbers and *refinement* of method. To these Paton adds a fourth, *responsibility* of the experimenter, a concern shared with the world outside the laboratory. Rowan and Paton agree that deliberate funding of research into methods for the relief of pain in animal experiments would be of advantage, and Rowan would like to see much more money available for the study of alternatives to animals. They agree that the LD₅₀ test (originally designed for the accurate assay, against a standard, of the toxicity of agents such as diphtheria toxin in the preparation of vaccines) has been misapplied to substances for which an approximate measure of toxicity, using fewer animals and causing less suffering, would be more appropriate. They agree also that the statutory requirements laid down by official bodies for testing drugs and other chemicals should not be allowed to go unchallenged, but that tests should be discontinued when superseded by methods using less animals, causing less suffering or dispensing with animals altogether.

The paradox is that, along with public concern with the plight of experimental animals, there is increasing demand for drugs, foods and household products to be "safe". Safety testing, for a long time to come, will involve the use of animals. Television and radio programmes often report exciting advances in medicine, surgery and biology based on animal experiments without drawing attention to the essential part of such experiments, together with a rational mixture of non-animal and human experiment, in achieving the advance. Sensational accusations hit the headlines of popular newspapers which seldom give coverage when such accusations have been shown to be without foundation. The truth, it seems, is not news.

The debate will continue. As Sir Peter Medawar says:

In the meantime we must grapple with the paradox that nothing but research on animals will provide us with the knowledge that will make it possible for us, one day, to dispense with the use of them altogether [*The Hope of Progress*; Methuen, 1972].

That debate will be all the better informed if the various protagonists read both of these books. □

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Honeyed history

John B. Free

The Archaeology of Beekeeping.

By Eva Crane.

Duckworth/Cornell University Press:
1984. Pp.360. £25, \$39.50.

THE history of man's relationship with the bee is a long and fascinating one; throughout the ages artists and poets have expressed appreciation of the bounty of the hive and the supposed virtue and selfless toil of the bee.

On the dust cover, the publisher claims that this is the first book "to explore the rich heritage of beekeeping archaeology". This is not true. However, earlier books in English that dealt exclusively with the history of beekeeping — among them Hilda M. Ransome's classic *The Sacred Bee in Ancient Times and Folklore* (of which a reprint is long overdue) and two works by H. Malcom Fraser, *Beekeeping in Antiquity and History of Beekeeping in Britain* — are no longer in print. During the past few years several other books have also included historical accounts of beekeeping. Nevertheless Dr Crane's book is a welcome and authoritative addition to the subject. Indeed it ranges more widely than its title implies and could more appropriately be called *A History of Beekeeping*.

Dr Crane provides detailed information on the latest archaeological and anthropological discoveries, notable among which are rock paintings depicting bee nests and honey-hunting in Zimbabwe, South Africa, Australia and central India. These and the long-known Spanish rock paintings are considered in relation to the honey-hunting techniques, still practised by primitive peoples in many parts of the world, to rob wild honeybee colonies of their honey and brood.

From Egyptian tomb paintings, ranging from 2400 to 600 BC, we obtain our first evidence of bees being kept in hives and of the harvesting and packing of honey into pottery vessels for later consumption. Horizontal stacked hives as depicted in the paintings are still to be seen today in Arab villages. Only a few Greek pottery hives and no Roman ones have been excavated; fortunately, however, Classical writers have left us with a wealth of information on their understanding of the honeybee colony and the types of beekeeping they practised.

During the past 2,000 years there has been a proliferation of hive types and methods of beekeeping, each adapted to local conditions. Illustrations and descriptions are given of clay pots, hollow logs, and wicker and straw baskets that are still used as hives in many parts of the world, and were undoubtedly used more widely in the past. Knowledge about primitive hives and beekeeping methods is not merely of

scholarly interest, as their use in modified form can be helpful in encouraging beekeeping in developing countries where there is often considerable potential for honey production. So it is crucial that we retain such information, and Dr Crane's book could be most helpful in furthering this end. For example, the evolution of hives with movable combs can be traced back to a Greek basket hive, which here deservedly receives considerable attention. The principles it embodies form the basis of a hive now used in East Africa and elsewhere.

Because most hives are of perishable material, few old ones have survived. However more durable material was used to form the shelters in which many of the early hives were placed. Some were kept in beehouses, each with its own access to the exterior; others in open-fronted shelters, and, most commonly in the United Kingdom, hives were kept individually in recesses — bee boles — in stone or brick

walls. Bee boles were most common in the eighteenth and nineteenth centuries, and the author obviously finds them particularly fascinating. She devotes 46 pages to discussion of bee boles and 33 pages to other hive shelters; the International Bee Research Association's records of the bee boles and shelters in the United Kingdom are given in an 80-page Appendix. While it is useful to have this information brought together in published form, it occupies nearly half of the book and so unbalances the presentation somewhat and gives it a bias towards the United Kingdom.

The book is attractively produced, contains much interesting and well-documented information, and many unusual illustrations. It will be a most worthy and useful addition to the beekeeping sections of libraries □

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Hope for an answer

William H. McNeill

Weapons and Hope.

By Freeman Dyson.

Harper & Row: 1984. Pp.340. \$17.26,
£10.95.

FREEMAN DYSON, whose last book set out to disturb the Universe, addresses this one to a more modest target: "... the gravest problem now facing mankind, the problem of nuclear weapons from a human rather than a technical point of view" (p.3). A human point of view means, above all, a moral point of view. But it is the morality of Odysseus rather than of Achilles, that is, of the cunning survivor, not the dead hero.

The author wanders back and forth between personal reminiscence, literary discourse and strategic discussion in a delightful if sometimes confusing way for a reader who seeks a rigorous argument about how to escape the threat of nuclear annihilation. Perhaps Dyson chose this approach to emphasize the human dimension. More likely it was because he recognizes logical weaknesses in his recipe for escape from our dilemma, but wishes to insist that hope — persistent and unrelenting hope — is the really important ingredient since only so can cheap despair, paralysis of will and atrophy of moral sense be forestalled.

Dyson wants a world without nuclear warheads. He asserts that with the appropriate exercise of moral indignation, political negotiation and technical investment in non-nuclear defensive systems the dismantling of the last nuclear device might be achieved after fifty years — with luck along the way. To reach this goal, American and Russian soldiers must be convinced that nuclear arms are counter-productive —

unusable in war, and debilitating to the morale of the armed services in peace. Only cooperation between what he calls "warriors" and "victims" can lead to the abolition of nuclear arms. The problem, then, is to find a concept of national defence that does not depend on nuclear weapons, and then attract a robust political following in support of the military policies such a concept implies.

Dyson discovers this in what he calls "live and let live", the principle that "We maintain the ability to damage you as badly as you can damage us, but we prefer our own protection to your destruction" (p.274). This rationale, Dyson thinks, would allow the United States to negotiate with the Russians for mutual reduction of existing nuclear weaponry all the way down to zero — perhaps. Holding to the idea of mutually assured destruction (plus planning on first use of nuclear tactical weapons in case of a Soviet attack in Europe), he says, will permanently prevent successful negotiation for the simple reason that Russian doctrine concerning the use of nuclear weapons differs from that of the United States, being aimed at survival against strategic rocketry, if need be by means of counterforce first strike.

I have difficulty in imagining just how Dyson's concept of "live and let live" could be implemented. His most surprising suggestion pertains to how American strategic rockets should be targeted:

The answer to this question is simple: We should not aim them at all. Live and let live excludes assured destruction targeting as inconsistent with the basic principle that live Americans are to be preferred to dead Russians. Limited nuclear war and counterforce targeting are excluded because they are crisis unstable, especially since we are assuming no change in the Soviet practice of counterforce targeting. What then are we to do with our strategic weapons? We must simply maintain them as best we can, making sure that they are as invulnerable as

possible to Soviet attack and that they are not aimed at anything in particular. This is what it means to say that we value them as bargaining chips but not as usable military weapons. [pp.275–276].

But aimed at nothing in particular is still aimed at something — what?

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Freeman Dyson — morality of Odysseus.

Technological development does alter war plans and can divert arms races into unforeseen paths. For example, miniaturization of hydrogen warheads drastically altered weaponry and war strategy in the 1950s and 1960s, as Dyson lucidly explains. Similar shifts in thinking may well arise in the future. New defensive weapons may make our ICBMs as obsolete as, for example, the disappearing-carriage coast artillery guns built so proudly about a century ago. But it is hard for me to imagine that new weapons will not be as destructive as those they displace. Persuading sovereign states and their military experts to substitute weaker weapons for those they have runs counter to the choices that have always been made in times past. Can the concept of “live and let live” really reverse that drift? Or is there only hope?

What I find odd about Dyson's approach is his acceptance of the existing state system as unalterable. Relocation of sovereignty is how other arms races have ended. If ours is ever to do so that seems a far more probable scenario than anything Dyson proposes. The fact that peace through subjugation is unpalatable may be why Freeman Dyson rejects this possibility without considering it. He seems to feel that it would be treason to his given name. But would it? Perhaps he should supplement Homer with Vergil, to see how empires may also find patriots and loyal subjects, profoundly grateful for the peace they bring. □

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Probable ideas

Cyril Domb

Statistical Physics and the Atomic Theory of Matter, from Boyle and Newton to Landau and Onsager.

By Stephen G. Brush.

Princeton University Press: 1983. Pp.356.

Hbk \$45, £38.70; pbk \$14.50, £12.70.

ELLIOTT Montroll, who died last December, was a leading figure in statistical physics for four decades. In a lecture delivered in 1958 entitled “One Hundred Years of Statistical Mechanics”, he argued that the field was initiated by the 1857 paper of Rudolf Clausius on “The Kind of Motion We Call Heat”. Admittedly there had been a good deal of activity since Newton, but activity is not the same as progress.

Montroll's thesis had the powerful backing of Willard Gibbs, who wrote in an obituary tribute to Clausius:

The origin of the kinetic theory of gases is lost in remote antiquity and its completion the most sanguine cannot hope to see. But a single generation has seen it advance from the stage of vague surmises to an extensive and well established body of doctrine. This is mainly the work of three men, Clausius, Maxwell, and Boltzmann of whom Clausius was the earliest in the field and has been called by Maxwell the principal founder of the science. We may regard his 1857 paper as marking his definite entry into this field, although many points were incidentally discussed in earlier papers.

Stephen Brush argues that it is the task of the historian of science to record activity as well as progress, to make sure that false starts and dead ends are given proper coverage as well as the dramatic and exciting successes. Therefore, in addition to describing the fundamental contributions of Clausius, Maxwell, Boltzmann and Gibbs, who laid the foundations of modern statistical physics, he also tells us in detail of the struggles of earlier pioneers such as Boyle, Newton, and Daniel Bernoulli, and of the unsuccessful wave theory of heat which was an attempt to emulate the triumphant wave theory of light.

Maxwell was the first to appreciate that the language of probability and statistics is essential in relating the behaviour of a macroscopic body to the properties of its microscopic constituents. Boltzmann developed and extended Maxwell's ideas and introduced his famous hypothesis relating the entropy of a macrostate to the combinatorial weight of microstates. He also pursued the kinetics of the approach to equilibrium, and was forced to grapple with the irreversibility paradox — how it is possible for equations of motion, which are reversible on the microscopic scale, to give rise to irreversible macroscopic phenomena. The introduction of these novel ideas and the battle to get them accepted are among the most fascinating stories in the history of physics. Brush con-

siders that these concepts may have helped significantly to bring about the acceptance of probability and indeterminacy in twentieth-century quantum physics.

Statistical mechanics played a central role in leading Planck to his famous quantum hypothesis. However Brush claims that the standard textbook account that the quantum of energy $h\nu$ was introduced to overcome the “ultra-violet catastrophe” is false and was invented by Paul Ehrenfest. Planck was looking for a formula which would fit the theoretical and experimental results on black body radiation. His result did not immediately produce much excitement in the world of physics. Gradually, as a result of Einstein's interpretation of the photoelectric effect, experimental developments in spectroscopy, and the success of Bohr's model of the atom in interpreting some of them, his new hypothesis gained acceptance.

A substantial part of the book is devoted to the history of modern fields of research, and here the author faces the difficult problem of coping with a burgeoning yet scattered literature. Many important results are first published in conference proceedings, and reliable guidance can be obtained only by consulting active researchers. Professor Brush has adopted this method, and has incidentally reproduced many interesting personal anecdotes.

The coverage given to different contemporary topics is subjective. The superfluidity of liquid helium receives exhaustive treatment, presumably because the author himself was actively engaged in the field. More space is devoted to this topic than to the whole of phase transitions and critical phenomena, each success or failure of the “collective excitation” approach of Landau and the rival “Bose Einstein condensation” of London and Tisza being faithfully recorded. By contrast the theory of superconductivity receives quite cursory treatment.

It is inevitable in a book covering such a wide variety of topics that there should be some omissions. For example, in the area of phase transitions (with which I myself have been concerned) the treatment of the solid-liquid transition makes no reference to the important work of Sir Francis Simon and his collaborators on the melting curve at high pressures. The concepts of scaling and universality which play such an important part in the modern theory of critical phenomena are not given proper attention. There is no account of the return to Landau's ideas in their Landau-Ginzburg form for the development of the renormalization group. The work of Patashinskii and Pokrovski in the Soviet Union should also be mentioned. In the classical period no reference is given to the work of Ornstein and Zernicke who provided the first correct description of correlations near the critical point; and Andrews's Bakerian Lecture to the Royal Society in 1869 deserves to be included in the

reference list. All these points might perhaps be taken into account in a second edition.

One of the tasks of the historian of science is to see that credit is properly assigned for scientific discoveries. Professor Brush emphasizes the part played by Wilhelm Lenz, the supervisor of Ernst Ising, in formulating the Ising model; in fact Brush uses the terminology "Lenz-Ising model". He also tells the sad story of J.J. Waterston who anticipated some of the ideas of Clausius in 1845. Waterston's paper was rejected by the Royal Society and the manuscript was not even returned to the author. In addition, the respective contributions of Bose and Einstein to the development of Bose-Einstein statistics, and of Fermi and Dirac to Fermi-Dirac statistics are described.

A useful feature of the book is the wide and detailed reference list, and research scientists will be surprised to find how the literature on the history of science has grown. For example, I learnt of the existence of English translations of several of Clausius's fundamental papers, and of a publication of Waterston's collected scientific papers. In all, this is a book from which all who are interested in statistical mechanics can benefit. □

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Managing disease

J.W. Deacon

The Nature and Practice of Biological Control of Plant Pathogens.

By R. James Cook and Kenneth F. Baker.

American Phytopathological Society: 1983. Pp. 539. \$43.

BIOLOGICAL control of plant diseases has generated much interest in recent years, in large part because of the efforts of a few workers such as James Cook and Kenneth Baker. The achievements up to 1974 were summarized and discussed in their earlier book, *Biological Control of Plant Pathogens*; this new work is a companion volume rather than a replacement, special attention being given to developments in the past decade. The book is impressive: quite simply, there has never been a better exposition of the "ecological" approach to plant disease control. The authors draw upon their first-hand experience and wide knowledge, and the result is an authoritative and highly readable account of the subject. It should be welcomed by both specialists and university students in the plant sciences.

The title, however, is surely misleading. In the view of many people, biological control is brought about by a third party (i.e. not by the host or the pathogen), either

applied directly or promoted by manipulating other factors. Instead, the authors have defined biological control so broadly that it leads to near-absurdities, for example when we are told (p.391) that the growing of genetically different crop cultivars in neighbouring areas is a form of biological control because it restricts the spread of pathogen races specific to any one cultivar. Further (p.407), the use of lime and inorganic fertilizers are said to be ways to achieve biological control if they affect plant resistance. The term then loses all useful meaning.

Semantics apart, this book is actually about *disease management* by manipulating the host, pathogen or environment (including microorganisms) to achieve what the authors term "sustainable agriculture". As such, it is timely and thought-provoking, though it could usefully have included chemical control methods, because a sustainable agriculture must harness, rationally, all available resources for plant protection. Let us hope that plant pathologists will follow the entomologists' lead in this respect.

Returning to biological control in a restricted sense, there are several successful techniques that are applied widely in commercial practice. But all too many of the "successes" discussed in this book have never reached the stage of exploitation, and the authors could have been more critical in this respect. For example, their earlier book cited astounding benefits when *Bacillus subtilis* was applied to seeds of crops in Australia: in field trials, carrot yields were increased by 48% and oat yields by 40%, even when the untreated crops showed no obvious disease symptoms. Baker was associated with this work, so now we could expect to learn of further developments. Instead, the earlier reports are merely repeated. Why aren't Australian growers using *B. subtilis* and getting huge yield increases? Was the work abandoned or were subsequent trials inconclusive? In order to justify its title, the book should have addressed such questions. And why cite yet more "successes" in the use of seed-applied microbes to control damping-off diseases? We knew this in the 1950s and there have been regular reports ever since, but the method has not been adopted commercially because we have cheap, dependable and reasonably safe fungicides that give the necessary short-term protection to seedlings.

In short, biological control will achieve more converts if it is *seen* to be approached with realism and less of an evangelical spirit. But this is a relatively minor point. Cook and Baker's new book will encourage and assist workers in this field, while helping to ensure that the momentum of work on biological control is sustained. □

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Clearly intermediate mechanics

J.W. Humberston

Theoretical Mechanics.

By E. Neal Moore.

Wiley: 1983. Pp. 456.

£28.10, \$37.90.

THERE are already so many books on classical mechanics, that one might well question the need for yet another — the field is not growing in the sense that fundamentally new material is continually being added to it. *Theoretical Mechanics* deserves attention, however, because the style of presentation and the philosophy behind it are somewhat unusual.

The book is designed for students who are already familiar with the subject matter covered in the usual undergraduate course in basic mechanics and who wish to have an introduction to more advanced topics such as Hamiltonian and Lagrangian mechanics, variational methods and canonical transformations. The author believes that an initial understanding of these topics is more likely to be acquired by practice in solving problems than by studying the formal structure of the subject. Accordingly, he has provided numerous worked examples and sets of problems at the end of each chapter.

The book begins with a brief review of basic Newtonian mechanics, and, in addition to the topics already mentioned, contains chapters on central force motion, rotations, oscillations and stability, and a brief introduction to the special and general theories of relativity. Several of these topics are treated in more elementary books on mechanics, but the presentation here is usually more advanced; for example, the Runge-Lenz vector is introduced in the discussion of the motion of a particle under an inverse square law of force.

Eighty pages are devoted to several mathematical appendices, some of which are rather inappropriate in a book at this level. So while the appendices on tensors and group theory are useful, it hardly seems necessary to give the elementary properties of vector operations, determinants and matrices. Finally, there is a quite comprehensive bibliography and a list of references to articles on mechanics published in the *American Journal of Physics*.

The book is well produced, contains very few typographical errors and is written in a pleasantly informal style. But, as the author himself admits in the preface, rigour is often sacrificed in the interests of clarity. □

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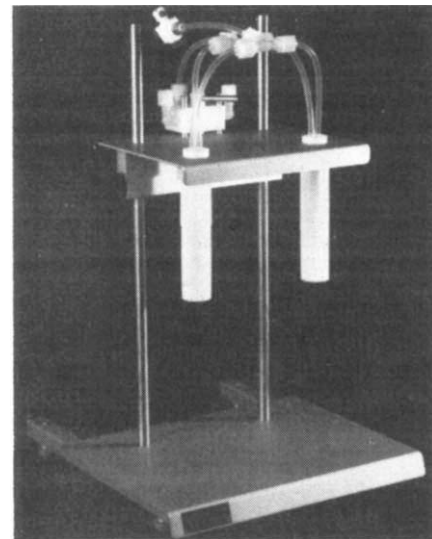
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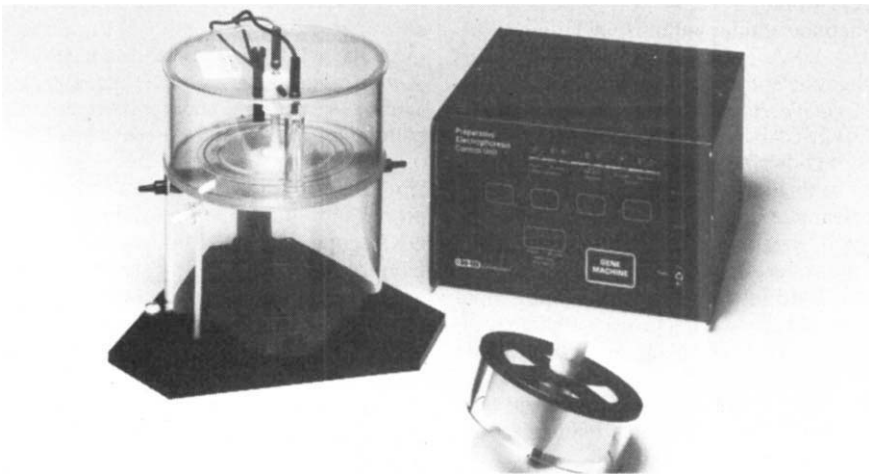
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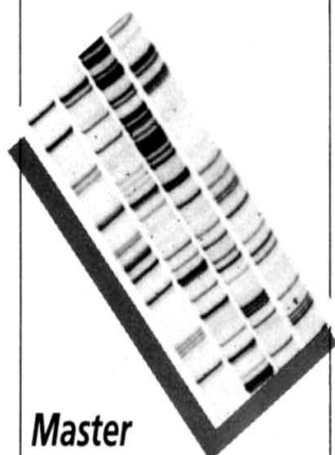
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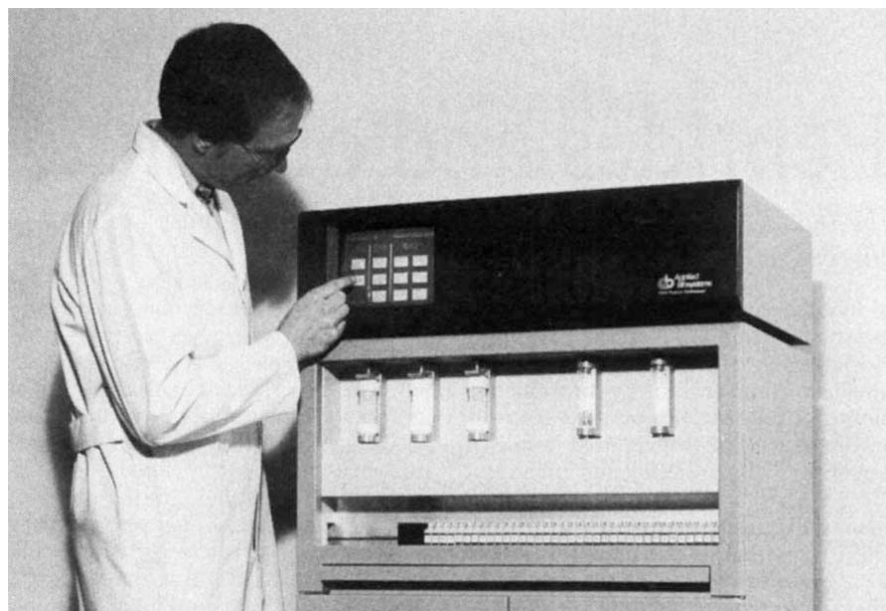
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